



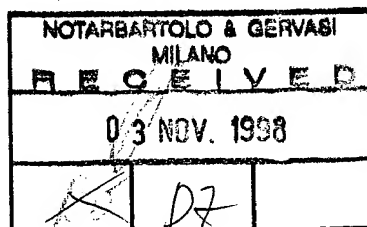
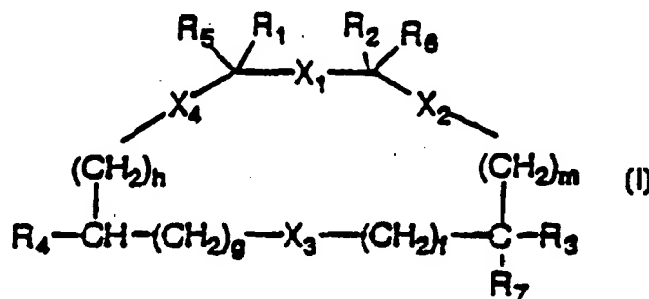
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(54) Title: MONOCYCLIC COMPOUNDS WITH FOUR BIFUNCTIONAL RESIDUES HAVING NK-2 ANTAGONIST ACTION

## (57) Abstract

The present invention refers to compounds of general formula (I) having NK-2 antagonist action, pharmaceutical compositions containing them, and processes for their preparation.



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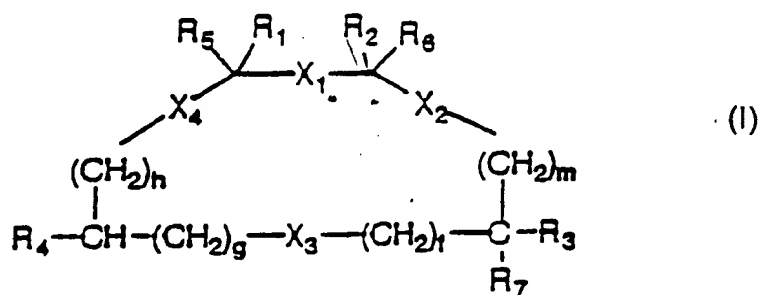
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# MONOCYCLIC COMPOUNDS WITH FOUR BIFUNCTIONAL RESIDUES HAVING NK-2 ANTAGONIST ACTION

## Scope of the invention

The present invention refers to new compounds having the general formula (I):



in which:

$X_1, X_2, X_3, X_4$ , which may be the same or different from one another, represent a group chosen from among -CONR-, -NRCO-, -OCO-, -COO-, -CH<sub>2</sub>NR-, -NR-CH<sub>2</sub>-, CH<sub>2</sub>-CH<sub>2</sub>-, where R is H or a C<sub>1-3</sub> alkyl or benzyl;

f, g, h, m, which may be the same or different from one another, represent a number chosen from among 0, 1 or 2;

$R_1$  and  $R_2$ , which may be the same or different from one another, represent a -(CH<sub>2</sub>)<sub>r</sub>-Ar group, where r = 0, 1, 2 and where Ar is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar group being possibly substituted with a maximum of 2 residues chosen from among C<sub>1-3</sub> alkyl or halo-alkyl, C<sub>1-3</sub> alkoxy, C<sub>2-4</sub> amino-alkoxy, halogen, OH, NH<sub>2</sub>, NR<sub>13</sub>R<sub>14</sub>, where R<sub>13</sub> and R<sub>14</sub>, which may be the same or different from one another, represent hydrogen or C<sub>1-3</sub> alkyl;

$R_3$  represents a group chosen from among:

- hydrogen

- linear or branched alkyl having the formula C<sub>n</sub>H<sub>2n+1</sub>, with n = 1-5, cyclo-alkyl or alkylcyclo-alkyl groups having the formula C<sub>n</sub>H<sub>2n-1</sub> with n = 5-9

- (CH<sub>2</sub>)<sub>r</sub>-Ar<sub>1</sub>, where r = 0, 1, 2 and where Ar<sub>1</sub> is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar<sub>1</sub> group being possibly substituted with a maximum of 2

residues chosen from among  $C_{1-3}$  alkyl or halo-alkyl,  $C_{1-3}$  alkoxy or amino-alkoxy, halogen, OH,  $NH_2$ ,  $NR_{13}R_{14}$ , where  $R_{13}$  and  $R_{14}$ , which may be the same or different from one another, represent hydrogen or  $C_{1-3}$  alkyl;

$R_4$  represents a group chosen from among:

5 - hydrogen or  $C_{1-6}$  alkyl

- L-Q, where L is a chemical bond or a linear or branched  $C_{1-6}$  alkyl residue and Q is a group chosen from among:

i) H, OH,  $OR_9$ ,  $NH_2$ ,  $NR_9R_{10}$ , guanidine, sulphate, phosphonate, phosphate, where  $R_9$  and  $R_{10}$ , which may be the same or different from one another, represent a hydrogen,  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxyalkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$ alkyl-CONHR<sub>12</sub>,  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or wherein  $R_9R_{10}$  joined together form with the N-atom a saturated 4-6 membered heterocycle possibly containing a further heteroatom chosen in the group consisting of N, O, S and wherein  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly protected with one or more  $C_{1-3}$ -acyl groups or substituted with amino-groups or  $C_{1-3}$ acylamino-groups;

ii) COOH, tetrazole,  $SO_2NH_2$ ,  $SO_2NHCOOR_8$ , CONHR<sub>8</sub>, NHCOR<sub>8</sub>, where  $R_8$  represents a linear or cyclic  $C_{1-6}$  alkyl chain containing one or more polar groups chosen from among the group: OH,  $NH_2$ ,  $NR_{15}R_{16}$ , COOH, CONHR<sub>12</sub>,  $PO_3H$ ,  $SO_3H$ ,  $OR_{11}$  and where  $R_{15}$  and  $R_{16}$ , which may be the same or different from one another, represent a hydrogen or  $C_{1-3}$  alkyl group, and where  $R_{11}$  is a  $C_{1-3}$  alkyl or  $C_{2-4}$  amino-alkyl chain,  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly protected with one or more  $C_{1-3}$ acyl groups or substituted with amino-groups or  $C_{1-3}$ acylamino-groups or  $R_{15}R_{16}$  joined together form with the N-atom a saturated 4-6 membered heterocycle possibly substituted with  $C_{1-3}$ alkyl-groups or with saturated 4-6 membered heterocycle-groups containing at least an N-atom;

iii) COOR<sub>17</sub>, CONHR<sub>12</sub>, OR<sub>12</sub> where  $R_{12}$  is a mono-, di- or tri-glycoside group possibly protected with one or more  $C_{1-3}$  acyl groups or substituted with amine or  $C_{1-3}$  acylamine groups and  $R_{17}$  is a group  $R_{12}$  as above defined or a group  $C_{1-3}$ alkyl,  $C_{1-3}$ alkylphenyl, wherein the phenyl-group can be substituted with a group OH,  $NO_2$ ,  $NH_2$ , CN,  $CH_3$ , Cl, Br;



R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, which may be the same or different from one another, represent a hydrogen or C<sub>1-3</sub> alkyl group.

Also included in the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions  
5 containing them.

In view of the presence of chiral centres in the compounds of formula (I), also the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

#### State of the art

10 The NK-2 receptor of tachykinins is widely expressed in the peripheral nervous system of mammals. One of the various effects produced by the selective stimulation of the NK-2 receptor is the contraction of smooth muscle. Hence antagonists of the NK-2 receptor may be considered agents capable of  
15 controlling excessive contraction of smooth muscle in any pathological condition in which the release of tachykinins concurs in the genesis of the corresponding disorder.

In particular, the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, kidney infections  
20 and colics may be considered conditions in which the administration of NK-2 antagonists may be effective (E.M. Kudlacz *et al.*, Eur. J. Pharmacol., 1993, 241, 17-25).

In addition, a number of NK-2 antagonists capable of surmounting the haemato-encephalic barrier have shown anxiolytic properties (D.M. Walsh *et al.*,  
25 *Psychopharmacology*, 1995, 121, 186-191).

Cyclic compounds, and in particular cyclic hexapeptides (A.T. McKnight *et al.*, Br. J. Pharmacol., 1991, 104, 355) and bicyclic hexapeptides (V. Pavone *et al.*, WO 93/212227) or cyclic hexapeptideptides (L. Quartara *et al.*, J. Med. Chem., 1994, 37, 3630; S.L. Harbeson *et al.*, *Peptides, Chemistry and Biology*.  
30 *Proceedings of the Twelfth American Peptide Symposium*, 1992, 124) are known in the literature for their antagonistic activity towards the NK-2 receptor of tachykinins.

It has now surprisingly been found that products of lower molecular weight, monocyclic ones, containing only four bifunctional residues linked together via peptide or pseudopeptide bond, present high pharmacological activity associated to a considerable selectivity for the human NK-2 receptor, and thus  
5 are proposed as valid alternatives.

Detailed description of the invention

The present invention therefore sets itself the aim of making available new monocyclic compounds containing four bifunctional residues linked together via peptide or pseudopeptide bonds having antagonistic action on the NK-2  
10 receptor, with the general formula (I), as defined previously.

Also forming part of the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions containing them.

In view of the presence of chiral centres in the compounds of formula (I), also  
15 the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

According to the invention preferred compounds of general formula (I) are those in which:

f, g, h, m, which may be the same or different from one another, may be 0 or 1;  
20  $R_1$  and  $R_2$ , which may be the same or different from one another, represent the side chain of a natural amino acid chosen from among tryptophan, phenyl alanine, tyrosine, histidine or the side chain of a non-natural amino acid chosen in the group:

tryptophan and phenyl alanine, either mono- or di-substituted with residues  
25 chosen from among  $C_{1-3}$  alkyl or halo-alkyl,  $C_{1-3}$  alkoxy or amino-alkoxy, halogen, OH,  $NH_2$ ,  $NR_{13}R_{14}$ , where  $R_{13}$  and  $R_{14}$ , which may be the same or different from one another, represent a hydrogen or  $C_{1-3}$  alkyl group;

$R_3$  represents a group chosen from among:

- linear or branched alkyl having the formula  $C_nH_{2n+1}$ , with  $n = 1-5$  (chosen in the  
30 group consisting of methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl) cycloalkyl or alkylcycloalkyl of formula  $C_nH_{2n-1}$  with  $n = 5-9$  (chosen in the group consisting of cyclopentyl, cyclohexyl, methylcyclohexyl)

-  $(CH_2)_r-Ar_1$ , where  $r = 1$  or  $2$  and where  $Ar_1$  is an aromatic group chosen from among:  $\alpha$ -naphthyl,  $\beta$ -naphthyl, phenyl, indole, the said  $Ar_1$  group being possibly substituted with a maximum of 2 residues chosen from among  $C_{1-3}$  alkyl,  $CF_3$ ,  $C_{1-3}$  alkoxy, Cl, F, OH,  $NH_2$ ;

5  $R_4$  represents an L-Q group where:

L is a chemical bond or  $CH_2$ , and

Q is a group chosen from among:

- OH,  $NH_2$ ,  $NR_9R_{10}$ ,  $OR_{11}$ , and where  $R_9$  and  $R_{10}$ , which may be the same or different from one another, represent a hydrogen or  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxy alkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$ alkyl- $CONHR_{12}$  wherein  $R_{12}$  is a monoglycosidic group derived from D or L pentoses or hexoses (chosen in the group consisting of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine and their N-acetylated derivatives),  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or wherein  $R_9R_{10}$  are joined together to form with the N atom a morpholine or a
- 15 piperidine ring and where  $R_{11}$  is a  $C_{1-3}$  alkyl chain, or a  $C_{2-4}$  amino-alkyl chain;
- $NHCOR_8$  wherein  $R_8$  is a cyclohexane containing from 2 to 4 OH groups, a  $C_{1-6}$  alkylchain containing a polar group (chosen in the group consisting of  $NH_2$ , COOH,  $CONHR_{12}$  (wherein  $R_{12}$  is as hereabove defined) or [1,4']bipiperidine)
- COOH,  $COOR_{17}$  or  $CONHR_{12}$ , wherein  $R_{12}$  is as hereabove defined and  $R_{17}$  is
- 20 as  $R_{12}$  or a group 4-nitrobenzyl and  $R_{12}$  is a monoglycosidic group derived from D or L pentoses or hexoses (chosen in the group consisting of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine and their N-acetylated derivatives).

-  $R_5$ ,  $R_6$ ,  $R_7$  are H.

25 Likewise preferred are isomers that present an R configuration on the carbon atom that carries the  $R_3$  and  $R_7$  substituents.

Pharmaceutically acceptable salts of compounds of formula (I) include the salts with inorganic acids (such as, hydrochloric acid, hydrobromic acid, hydrogen iodide, sulphuric acid, nitric acid, phosphoric acid) and organic acids (such as,

30 acetic, propionic, succinic, malonic, citric, tartaric, metasulphonic, *para*-toluenesulphonic acids), as well as salts of pharmaceutically acceptable bases, both inorganic (such as, hydroxides of sodium, potassium, calcium,

magnesium, zinc and aluminium) and organic bases (such as, amines like methyl amine, diethyl amine, triethyl amine, ethyl amine, tromethamine or piperidine).

According to the invention, the compounds of formula (I) containing peptide or  
5 pseudopeptide bonds may be obtained by means of classical condensation methods using techniques known in the literature. The general method chosen by us for preparing the peptide compounds ( $X_1$ - $X_4$  = -CONR-, -NRCO-) involves the synthesis in solution of the linear peptide chain using amino acids, dicarboxyl or diamine derivatives suitably protected and, after selective  
10 deprotection of the C- and N-terminals, cyclization in polar organic solvents in diluted solution. As method of activation of the carboxyl groups, that using PyBOP and DIEA in DMF or HBT and EDC in DMF are generally preferred.

To provide an example, the attached diagram presents the general synthesis of compounds of formula (I) in which  $X_1 = X_2 = X_3 = X_4$  = -CONH-.

15 The dicarboxyl precursors 7 containing the  $R_4$  group, and the diamine precursors 4 containing the  $R_3$  and  $R_7$  groups were prepared using methods described in the literature.

In particular, the synthesis of the succinic derivatives, with  $R_4$  = alkyl or  $(CH_2)_n$ -Ar, is described by R. Conrow *et al.*, J. Org. Chem., 1986, 51, 938 and by S.G.  
20 Cohen *et al.*, J. Am. Chem. Soc., 1968, 90, 3495, whilst in the case of  $R_4$  = H, amine group, hydroxyl group or carboxyl group, the following were respectively used: succinic anhydride, aspartic acid, malic acid or carboxysuccinic acid appropriately protected.

The synthesis of the ethylene diamine derivatives containing the  $R_3$ ,  $R_7$  groups  
25 was performed starting from the corresponding N-protected amino acids by reduction of the carboxyl to alcohol with  $BH_3$ .THF (C.F. Stanfield *et al.*, J. Org. Chem., 1981, 46, 4797, 4799; I.R. Ollmann *et al.*, Bioorg. Med. Chem., 1995, 3, 969), conversion to azide via the corresponding mesylate and subsequent reduction to amino group (P.G. Mattingly, Synthesis, 1990, 366; P.M. O'Brien *et al.*, J. Med. Chem., 1994, 37, 1810).  
30

The compounds containing reduced peptide bonds ( $X_1$ - $X_4$  = -CH<sub>2</sub>-NR-, -NR-CH<sub>2</sub>-) were synthesized in solution according to known methods, such as

reductive amination of the aldehyde of an amino acid with the amine function of a protected amino acid or peptide, in the presence of  $\text{NaCNBH}_4$  as reducer in DMF/AcOH (K.A. Jacobson *et al.*, J. Med. Chem., 1983, 26, 492; R.F. Borch *et al.*, J. Am. Chem. Soc., 1971, 93, 2897; J.P. Salvi *et al.*, Tetr. Lett., 1994, 35, 1181). The aldehydes were obtained by reduction with  $\text{LiAlH}_4$  of the  
5 corresponding protected amino acids, N,O-dimethylhydroxy-amates according to the method described by J.A. Feherentz *et al.*, Synthesis, 1983, 676 and Int. J. Peptide Res., 1985, 26, 236.

The compounds of formula 10 wherein  $\text{R}_4$  is  $\text{NH}_2$  or  $\text{COOH}$  can be derivatized  
10 into compounds of formula 1 wherein  $\text{R}_4$  is  $\text{NR}_9\text{R}_{10}$ , guanidine, tetrazole,  $\text{NHCOR}_8$ ,  $\text{CONHR}_8$ ,  $\text{COOR}_{17}$ ,  $\text{CONHR}_{12}$ , wherein  $\text{R}_9$ ,  $\text{R}_{10}$ ,  $\text{R}_8$ ,  $\text{R}_{12}$  and  $\text{R}_{17}$  are as above defined, according to known methods.

The compounds of formula (I) as specified above have proved to be powerful  
15 antagonists of the NK-2 receptor of tachykinins, and hence can be administered as agents capable of controlling any central or peripheral manifestation due to excessive activation of tachykinergic neurons, and in particular excessive contraction of smooth muscle in any pathological condition in which release of tachykinins concurs in the genesis of the corresponding disorders.

In particular, the bronchospastic and inflammatory component of asthma, of  
20 coughing, of pulmonary irritation, intestinal spasms, spasms of the biliary tract, and local spasms of the bladder and ureter in the course of cystitis and kidney infections and colics may be considered conditions in which the administration of the compounds of formula (I), as NK-2 antagonists, may prove effective.

The use as anxiolytic agents should also be considered for those compounds  
25 that are provided with the appropriate chemico-physical characteristics for penetration into the CNS.

The compounds of formula (I) that are the subject of the present invention are suited for administration for therapeutic purposes to higher animals and man through the parenteral, oral, inhalational and sublingual routes, achieving  
30 pharmacological effects according to the properties described above. For administration through parenteral (intravenous, intramuscular, and intradermal) routes, sterile or lyophilized preparations are used. As far as the nasal,

inhalational and sublingual instillation routes are concerned, aqueous solutions, aerosol preparations, powders or capsules are used according to the particular case.

The doses of active principle in the aforesaid compositions may range between  
5 0.02 and 10 mg/kg of body weight.

The invention will now be illustrated in the examples that follow, which, however, have no limiting effect.

#### Example 1

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

10 (compound of formula (I) where: X<sub>1</sub> = X<sub>2</sub> = X<sub>3</sub> = X<sub>4</sub> = -CO-NH-; R<sub>1</sub> = -CH<sub>2</sub>-(indol-3-yl); R<sub>2</sub> = R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>; R<sub>4</sub> = R<sub>5</sub> = R<sub>6</sub> = R<sub>7</sub> = H; m = h = 0, f = g = 1; the carbon atoms C-R<sub>1</sub> and C-R<sub>2</sub> have an S configuration, whereas C-R<sub>3</sub> has an R configuration)

#### a) Synthesis of BOC-Trp-Phe-OH dipeptide

15 Di-*tert*-butyl carbonate (3.4 g) was added to a solution of H-Trp-Phe-OH (5 g) in dioxane (30 ml), H<sub>2</sub>O (15 ml) and NaOH 1M (15.6 ml), cooled to 0-5°C under stirring. The reaction mixture was kept stirred for 2 hours, and then concentrated and extracted with pentane (2 x 20 ml). The aqueous phase was cooled with ice, with the addition of AcOEt (50 ml), KHSO<sub>4</sub> to obtain pH 2-3,  
20 separated and extracted with AcOEt (2 x 50 ml). The re-united organic phases were washed with brine (50 ml), vacuum dried and evaporated at 30°C to obtain 6 g of the desired compound as a white semi-solid residue.

TLC: r.f. 0.55 (chloroform / cyclohexane / AcOH / H<sub>2</sub>O = 45 / 45 / 5 / 5), 0.52 . (CHCl<sub>3</sub> / MeOH = 9/1)

#### 25 b) Synthesis of (R)-1-benzyl-2-benzyloxycarbonylamino-ethyl amine

The synthesis was carried out following the method described by P.G. Mattingly, Synthesis, 1990, 366, starting from BOC-D-phenylalaninol

#### c) Synthesis of BOC-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-Z] (5)

(R)-1-benzyl-2-benzyloxycarbonylamino ethyl amine (750 mg), PyBOP (1.37 g),  
30 and DIEA (0.9 ml) were added to a solution of BOC-Trp-Phe-OH (1.19 g, 2.63 mmol.) in anhydrous DMG (10 ml) under nitrogen.

The reaction mixture was kept stirred overnight at room temperature, AcOEt (80 ml) was added, and the mixture was washed with HCl 1N (3 x 30 ml), Na<sub>2</sub>CO<sub>3</sub> 5% (3 x 30 ml), and H<sub>2</sub>O (30 ml). The organic phase was vacuum evaporated at 30°C to obtain 1.8 g of an ivory-coloured solid residue.

- 5 The crude compound was purified by washing in suspension with AcOEt under heat and with MeOH at room temperature to obtain 1.15 g of the desired product 5 as a white solid. MS(TS) : [MH<sup>+</sup>] = 718

d) Synthesis of H-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-Z] (6)

- TFA (6 ml) was added, under stirring and at room temperature, to a suspension  
10 of the compound 5 (1.1 g) in CHCl<sub>3</sub> (30 ml), and a clear solution was seen to form immediately. The reaction mixture was kept stirred for 1.5 hours, and the disappearance of the precursor was monitored by means of HPLC analysis. After evaporation of the solvent, the residue was diluted with AcOEt (100 ml), washed with NaHCO<sub>3</sub> 5% (2 x 30 ml) and brine (30 ml).

- 15 The organic phase was dried with MgSO<sub>4</sub> and vacuum evaporated at 30°C. The solid residue was purified by means of flash-chromatography (CHCl<sub>3</sub>/MeOH = 95/5) to obtain 821 mg of the desired compound 6 as a white solid.

TLC: r.f. 0.50 (CHCl<sub>3</sub>/MeOH = 9/1)

- 20 e) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-Z] (compound 8 where: PG<sub>2</sub> = OH; PG<sub>1</sub> = Z)

- NEt<sub>3</sub> (0.095 ml) and succinic anhydride (68 mg) were added to a solution of compound 6 (420 mg) in anhydrous DMF (10 ml) under stirring and at room temperature. The reaction mixture was kept stirred at room temperature for 4  
25 hours.

After evaporation of the solvent, the residue was suspended in H<sub>2</sub>O and kept stirred for 5 minutes. The solid was filtered and washed in suspension twice using MeOH to obtain 242 mg of the desired compound 8 as a white solid.

TLC: r.f. 0.50 (CHCl<sub>3</sub>/MeOH = 8/2)

- 30 f) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH<sub>2</sub>] (9)

The compound 8 (225 mg) was suspended in MeOH (10 ml) and hydrogenated in the presence of Pd/C 10% (50 mg) at atmospheric pressure and room

temperature. HPLC analysis after 4 hours showed that the precursor had disappeared completely.

The catalyst was filtered and washed with MeOH. After evaporation of the solvent, 158 mg of the desired compound 9 were obtained as a white solid.

5 m.p. = 142-4°C; TLC: r.f. 0.70 (*n*-butanol / AcOH / H<sub>2</sub>O = 6 / 2 / 2)

g) Synthesis of cyclo{Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}(10)

PyBOP (145 mg) and DIEA (0.09 ml) were added to a solution of compound 9 (148 mg) in anhydrous DMF (5 ml) stirred under nitrogen.

10 The reaction mixture was kept stirred for 5 hours and, after evaporation of the solvent, the residue was suspended in AcOEt, kept stirred for 10 minutes, and filtered, to obtain 100 mg of a solid product.

Part of the product (50 mg) was purified by HPLC to obtain 18 mg of the desired compound 10 as a white solid.

15 MS (TS) : [MH<sup>+</sup>] = 566; 1H-NMR (DMSO): d 2.15-2.35 (m, 2H), 2.55-2.85 (m, 8H), 2.96-3.04 (m, 2H), 3.90-4.02 (m, 1H), 4.03-4.15 (m, 1H), 4.25-4.42 (m, 1H), 6.71 (d, 1H), 6.90-7.42 (m, 16H), 8.09 (m, 1H), 8.50 (d, 1H), 10.82 (s, 1H).

Following the procedure described in Example 1, the compounds specified below were obtained:

#### Example 2

20 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

(compound of formula I in which the substituents are defined as in Example 1, but all the C-R<sub>1</sub>, C-R<sub>2</sub> and C-R<sub>3</sub> atoms have an S configuration) 1H-NMR (DMSO): d 1.95-2.32 (m, 2H), 2.34-2.90 (m, 6H), 2.92-3.18 (m, 2H), 3.60-3.82 (m, 1H), 4.00-4.40 (m, 4H), 6.90-7.36 (m, 14H), 7.39-7.54 (m, 2H), 7.64 (d, 1H),  
25 7.88 (t, 1H), 8.27 (d, 1H), 10.78 (s, 1H).

#### Example 3

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>11</sub>)-CH<sub>2</sub>-NH-]}

(compound of formula I in which X<sub>1</sub> = X<sub>2</sub> = X<sub>3</sub> = X<sub>4</sub> = CO-NH-; R<sub>1</sub> = -CH<sub>2</sub>-(indol-3-yl); R<sub>2</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>; R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>11</sub>; R<sub>4</sub> = R<sub>5</sub> = R<sub>7</sub> = H; m = h = 0, f = g = 1;  
30 the carbon atoms C-R<sub>1</sub> and C-R<sub>2</sub> have an S configuration, whereas C-R<sub>3</sub> has an R configuration) 1H-NMR (DMSO): d 0.65-0.95 (m 2H), 1.00-1.38 (m, 6H), 1.45-1.75 (m, 5H), 2.05-2.30 (m, 2H), 2.40-2.85 (m, 6H), 3.20-3.60 (m, 1H), 3.61-



3.78 (m, 1H), 3.80-3.96 (m, 1H), 3.98-4.10 (m, 1H), 4.38-4.55 (m, 1H), 8.47 (d, 1H), 6.90-7.45 (m, 11H), 8.02 (m, 1H), 8.47 (d, 1H), 10.78 (d, 1H).

#### Example 4

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>(4-OCH<sub>3</sub>))-CH<sub>2</sub>-NH-]}

- 5 (compound of formula I in which X<sub>1</sub> = X<sub>2</sub> = X<sub>3</sub> = X<sub>4</sub> = CO-NH-; R<sub>1</sub> = -CH<sub>2</sub>-(indol-3-yl); R<sub>2</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>; R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>(4-OCH<sub>3</sub>); R<sub>4</sub> = R<sub>5</sub> = R<sub>6</sub> = R<sub>7</sub> = H; m = h = 0, f = g = 1; the carbon atoms C-R<sub>1</sub> and C-R<sub>2</sub> have an S configuration, whereas C-R<sub>3</sub> has an R configuration) 1H-NMR (DMSO): d 2.13-2.37 (m, 2H), 2.50-2.85 (m, 8H), 3.25-3.50 (m, 1H), 3.58-3.80 (m, 4H), 3.85-4.00 (m, 1H), 4.02-4.18 (m, 1H), 4.28-4.45 (m, 1H), 6.65-7.47 (m, 16H), 8.02-8.16 (m, 1H), 8.48 (d, 1H), 10.80 (s, 1H).

#### Example 5

Cyclo{-Suc-Trp(5F)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

- 15 (compound of formula I, in which R<sub>1</sub> = -CH<sub>2</sub>-(5-fluoroindol-3-yl), and the other substituents are as defined in Example 1) MS(ES+): [MH<sup>+</sup>]=584

#### Example 6

Cyclo{-Suc-Trp(Me)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

- (compound of formula I, in which R<sub>1</sub> = -CH<sub>2</sub>-(N-methylindol-3-yl), and the other substituents are as defined in Example 1) MS(ES+): [MH<sup>+</sup>]= 580

#### Example 7

Cyclo{-Suc-Phe(3,4-Cl)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

- 20 (compound of formula I, in which R<sub>1</sub> = -(3,4-dichlorobenzyl), and the other substituents are as defined in Example 1) MS(ES+): [MH<sup>+</sup>]=595

#### Example 8

25 Cyclo{-Suc-Trp-Phe(3,4-Cl)-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

- (compound of formula I, in which R<sub>2</sub> = -(3,4-dichlorobenzyl), and the other substituents are as defined in Example 1) (MS(ES+): [MH<sup>+</sup>]= 634

#### Example 9

Cyclo{-Suc-Trp-Tyr-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

- 30 (compound of formula I, in which R<sub>2</sub> = -(4-hydroxybenzyl), and the other substituents are as defined in Example 1) (MS(ES+): [MH<sup>+</sup>]= 582

#### Example 10

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>-3,4-diCl)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which R<sub>3</sub> = -(3,4-dichlorobenzyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 634

Example 11

5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-4-OH)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which R<sub>3</sub> = -(4-hydroxybenzyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 582

Example 12

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

10 (compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>-CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>, and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 580

Example 13

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-2-naphthyl)-CH<sub>2</sub>-NH-]}

15 (compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>-(2-naphthyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 616

Example 14

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-indol-3-yl)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>-(indol-3-yl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 605

20 Example 15

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-5-F-indol-3-yl)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>-(5-fluoroindol-3-yl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 623

Example 16

25 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-3-F)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-3-F, and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 584

Example 17

Cyclo{-Suc-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>-3,4-diF-CH<sub>2</sub>-NH)-]}

30 (compound of formula (I) wherein R<sub>3</sub> = -(3,4-difluorobenzyl) and the other substituents are as defined in Example 1 MS (ES+): [MH+]= 602

Example 18

Cyclo{-Suc-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-CF<sub>3</sub>-CH<sub>2</sub>-NH)-]}

(compound of formula (I) wherein R<sub>3</sub> = -(4-trifluoromethylbenzyl) and the other substituents are as defined in Example 1) MS (ES<sup>+</sup>): [MH<sup>+</sup>] = 634

Example 19

5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-NH-]}

(compound of formula (I) where: X<sub>1</sub> = X<sub>2</sub> = X<sub>3</sub> = X<sub>4</sub> = -CO-NH-; R<sub>1</sub> = -CH<sub>2</sub>-(indol-3-yl); R<sub>2</sub> = R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>; R<sub>4</sub> = R<sub>5</sub> = R<sub>6</sub> = R<sub>7</sub> = H; f = h = 0; m = g = 1; the carbon atoms C-R<sub>1</sub> and C-R<sub>2</sub> have an S configuration, whereas C-R<sub>3</sub> has an R configuration)

10 a) Synthesis of (R)-2-*tert*-butoxycarbonylamino-3-phenyl-propylamine

The synthesis was performed according to the method described by P.G. Mattingly, Synthesis, 1990, 366, starting from BOC-D-phenylalaninol.

b) Synthesis of Z-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-NH-BOC] (5)

(R)-2-*tert*-butoxycarbonylamino-3-phenyl-propylamine (titre 65%, 1.1 g), PyBOP (1.45 g), and DIEA (0.98 ml) were added to a solution of Z-Trp-Phe-OH (1.4 g) in anhydrous DMF (15 ml) under nitrogen. The reaction mixture was kept stirred overnight at room temperature, AcOEt (100 ml) was added, and the mixture was washed with HCl 1N (3 x 30 ml), Na<sub>2</sub>CO<sub>3</sub> 5% (3 x 30 ml), and H<sub>2</sub>O (30 ml). During the washings, the product partly precipitated, and was filtered and re-  
20 united to the organic phase. After vacuum evaporation of the solvent, the residue (2.4 g) was washed in suspension with AcOEt and vacuum dried on P<sub>2</sub>O<sub>5</sub>, to obtain 1.79 g of the desired compound 5 as a white solid.

TLC: r.f. 0.86 (CHCl<sub>3</sub>/MeOH = 95/5); r.f. 0.78 (AcOEt)

c) Synthesis of H-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-NH-BOC] (6)

25 A suspension of the compound 5 (1.7 g) in MeOH (350 ml) was hydrogenated in the presence of Pd/C 10%, at atmospheric pressure and room temperature, until the precursor disappeared (HPLC analysis). After elimination of the catalyst by filtration and vacuum evaporation of the solvent, the residue was washed in suspension with AcOEt to obtain 890 mg of the desired compound 6  
30 as a white solid.

TLC: r.f. 0.38 (CHCl<sub>3</sub>/MeOH = 9/1), r.f. 0.26 (AcOEt)

d) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-NH-BOC] (compound 8 where: PG<sub>2</sub> = OH; PG<sub>1</sub> = BOC)

Succinic anhydride (158 mg) and NEt<sub>3</sub> (0.21 ml) were added to a solution of compound 6 (840 mg) in anhydrous DMF (20 ml) under nitrogen. The reaction mixture was kept stirred at room temperature overnight. After vacuum evaporation of the solvent at a temperature of 30°C, the residue was treated with H<sub>2</sub>O at 40-50°C, filtered, washed in suspension with MeOH (15 ml), and vacuum dried to obtain 600 mg of the desired compound 8 as a white solid.

TLC: 0.63 (CHCl<sub>3</sub>/MeOH = 8/2)

10 e) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-NH<sub>2</sub>]• TFA (9 TFA)

TFA (2ml) was added, under stirring, to a suspension of compound 8 (560 mg) in CH<sub>2</sub>Cl<sub>2</sub> (15 ml), and a clear solution was obtained. After 2 hours at room temperature, the solvent was evaporated, and the residue diluted with ether, 15 filtered and dried to obtain 500 mg of the desired compound 9 TFA as an ivory-coloured solid.

TLC: 0.58 (CHCl<sub>3</sub>/MeOH = 8/2), 0.74 (*n*-butanol/AcOH/H<sub>2</sub>O = 6/2/2)

f) Synthesis of cyclo{-Suc-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-NH-]} (10)

PyBOP (447 mg), and DIEA (0.37 ml) were added, under nitrogen, to a solution of 9 TFA (500 mg) in anhydrous DMF (20 ml). The reaction mixture was kept stirred overnight at room temperature. After evaporation of the solvent, the residue was washed in suspension with citric acid 5% and H<sub>2</sub>O. The product was dried on P<sub>2</sub>O<sub>5</sub>, washed in suspension using AcOEt and MeOH under heat, to obtain 110 mg of a solid. A portion was purified by HPLC to obtain 25 mg of 25 the desired compound 10 as a white solid.

<sup>1</sup>H-NMR (DMSO): δ 2.10-2.40 (m, 4H), 2.45-2.58 (m, 1H), 2.60-3.05 (m, 7H), 3.80-3.90 (m, 1H), 3.92-4.05 (m, 1H), 4.20-4.38 (m, 1H), 6.90-7.40 (m, 16H), 7.52-7.58 (m, 1H), 8.11 (d, 1H), 8.37 (d, 1H), 10.79 (s, 1H).

#### Example 20

30 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-NH-]}

(compound of formula I in which the substituents are defined as in Example 19, except for the fact that C-R<sub>3</sub> has an S configuration).

The compound was obtained following a procedure similar to that described for Example 19.

<sup>1</sup>H-NMR (DMSO):  $\delta$  1.98-2.26 (m, 2H), 2.40-2.88 (m, 8H), 2.98-3.11 (m, 1H), 3.66-3.84 (m, 1H), 3.98-4.23 (m, 2H), 4.40-4.58 (m, 1H), 6.89-7.48 (m, 17H), 8.10 (d, 1H), 8.44 (d, 1H), 10.83 (s, 1H).

Proceeding in a similar way as that described in Example 1 above, the following compounds were obtained:

#### Example 21

Cyclo{-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]-(CH<sub>2</sub>)<sub>3</sub>CO-}

(compound of formula I, in which R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub> and X<sub>3</sub> = -CH<sub>2</sub>-NH-, and the other substituents are as defined in Example 1. MS (ES<sup>+</sup>): [MH<sup>+</sup>]=552.

#### Example 22

Cyclo{-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-N(CH<sub>3</sub>)]-(CH<sub>2</sub>)<sub>3</sub>CO-}

(compound of formula I, wherein R<sub>3</sub> = -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub> and X<sub>3</sub> = -CH<sub>2</sub>N(CH<sub>3</sub>)- and the other substituents are as defined in Example 1. MS(ES<sup>+</sup>):[MH<sup>+</sup>]=566.

#### EXAMPLE 23

Cyclo{-Suc[1(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH-]}

(compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NH<sub>2</sub> and the other substituents are as defined in Example 1 while the carbon atom C-R<sub>4</sub> has configuration S).

a) Synthesis of Boc-Asp[Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-Z]-OBz]

(compound 8 wherein: PG<sub>2</sub> = OBzl, PG<sub>1</sub> = Z

To a solution of compound 6 (see Example 1d) (650 mg) in anhydrous DMF (30 ml) Boc-Asp-OBzl (340 mg), PyBOP (656 mg) and ET<sub>3</sub>N (0.4 ml) are added under stirring at room temperature. The mixture is stirred for 2 h at room temperature. After evaporation of the solvent under vacuum the residue was treated with H<sub>2</sub>O giving a solid residue which is filtered, washed with water and dried. The solid was recrystallized from ethanole giving 640 mg of the desired compound 8 in the form of a white solid.

MS (ES<sup>+</sup>): [MH<sup>+</sup>]=923; HPLC performed in the following conditions: silica column C<sub>18</sub> particles size 5 $\mu$ m and pores of 100 Å (analytical data: 20% carbon and C<sub>18</sub> Surface Coverage 3.3  $\mu$ moles/m<sup>2</sup>), length: 3.9x150mm; mobile phase

having a linear gradient of acetonitrile containing 0.1%(v/v) TFA (phase B) against aqueous TFA 0.1% (v/v) (phase A), from 20% to 80% in B in 20 minutes at a flux of 3 ml/min; determination by UV at 220 nm. Retention time:  $R_t = 21.1$  min.

5 b) Synthesis of Boc-Asp[Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH<sub>2</sub>]-OH (9)

The compound 8 (of Example 23a) (600 mg) was solved in DMF (2 ml) and diluted with MeOH (30 ml), hydrogenated in the presence of Pd/C 10% (100 mg) at room pressure and temperature for 5 h. The catalyser was filtered and washed with MeOH. After evaporation of the solvent 420 mg of the desired product 9 were obtained in the form of a white solid.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]= 663; HPLC (same conditions as above):  $R_t = 11.07$ .

c) Synthesis of cyclo{-Suc[1(S)NH-BOC]-Trp-Phe[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} (10)

To a solution of compound 9 (see example 23b) (7.2 g) in anhydrous DMF (900 ml) 4 g of HBT and 2 g of EDC were added under stirring and nitrogen atmosphere. The mixture was stirred for 5 h and, after evaporation of the solvent, the residue was treated with an aqueous solution of KHSO<sub>4</sub> 5% and extracted in ethylacetate.

The organic phase was washed with brine, NaHCO<sub>3</sub> 5% and again with brine, dried and evaporated the yellow solid obtained (5.2 g) was crystallized from isopropanol/water: 1/1 giving 3.2 g of a white solid. MS(ES<sup>+</sup>):[MH<sup>+</sup>]=681; HPLC (same conditions as above):  $R_t = 14.8$ .

d) Synthesis of cyclo{-Suc[(1(S)NH<sub>2</sub>)-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} (10)

To a suspension of compound 10 (see example 23d) (1g) in CH<sub>2</sub>Cl<sub>2</sub> (20 ml) TFA (7 ml) was added under stirring at 0°C giving a clear solution; thereafter the temperature is raised up to room temperature. The mixture was left at room temperature for 90 minutes and then the solvent was evaporated and the residue was treated with NaHCO<sub>3</sub> and water and extracted in ethylacetate. The organic phase was washed with brine, dried and evaporated giving a solid (800 mg).

MS(ES<sup>+</sup>):[MH<sup>+</sup>]= 581; HPLC (same conditions as above said):  $R_t = 9.4$ .

A sample of 20 mg is purified by HPLC giving 15 mg of trifluoroacetate: cyclo{-Suc[1(S)NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.TFA (10 TFA)

MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 581; HPLC: Rt = 9.4 (same conditions as above); <sup>1</sup>H-NMR (DMSO): δ 2.60-2.90 (m, 8H), 3.05-3.11 (m, 1H), 3.63-3.71 (m, 1H), 4.07-4.13 (m, 3H), 4.32-4.38 (m, 1H), 6.90-7.45 (m, 17H), 8.07 (bs, NH<sub>3</sub><sup>+</sup>), 8.22-8.28 (m, 1H), 8.57 (d, 1H), 10.82 (s, 1H).

Following the procedure described in Example 23 the following compounds were obtained:

Example 24

- 10 Cyclo{-Suc[1(R)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}  
(compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NH<sub>2</sub> and the other substituents are as defined in Example 1 while the carbon atom C-R<sub>4</sub> has configuration R) MS (ES<sup>+</sup>): [MH<sup>+</sup>] = 581

Example 25

- 15 Cyclo{-Suc[2(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}  
(compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NH<sub>2</sub> and the other substituents are as defined in Example 1 while the carbon atom C-R<sub>4</sub> has configuration S) MS (ES<sup>+</sup>): [MH<sup>+</sup>] = 581

Example 26

- 20 Cyclo{-Suc[2(R)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}  
(compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NH<sub>2</sub> and the other substituents are as defined in Example 1 while the carbon atom C-R<sub>4</sub> has configuration R) MS (ES<sup>+</sup>): [MH<sup>+</sup>] = 581

Example 27

- 25 Cyclo{-Suc[1(S)-NH(CH<sub>3</sub>)]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}  
(compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NH(CH<sub>3</sub>) and the other substituents are as defined in Example 1 while the carbon atom C-R<sub>4</sub> has configuration S) MS (ES<sup>+</sup>): [MH<sup>+</sup>] = 595

Example 28

- 30 Cyclo{-Suc[1-COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}

(compound of formula I wherein  $h = 1$ ,  $g = 0$ ,  $R_4 = -\text{COO}(\text{CH}_2-\text{C}_6\text{H}_4-4-\text{NO}_2)$  and the other substituents are as defined in example 23) (diastereoisomeric mixture in respect of C- $R_4$  and separation of the two epimers).

a) Synthesis of Boc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH<sub>2</sub>]

- 5 The Boc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-Z] (5 see example 1c) (1.2 g) was dissolved in a mixture of DMF (30 ml) and MeOH (200 ml) and hydrogenized in the presence of Pd/C 10% (200 mg) at room pressure and temperature, for 4 h. The catalyser was filtered and washed with MeOH, the solvent evaporated giving 700 mg of solid residue.

10 MS(ES<sup>+</sup>): [MH<sup>+</sup>] 584; HPLC (conditions of example 23): Rt = 11.1

b) Boc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-COCH[COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]CH<sub>2</sub>COO-tBu

- 424 mg of 2-(4-nitro-benzyloxycarbonyl)-succinic acid 4-tert-butyl ester were dissolved in DMF (20 ml). To the mixture HOBT (490 mg), EDC and Boc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH<sub>2</sub>] were added at 0°C under stirring; the temperature was raised to room temperature while stirring for 2 h. The solvent was evaporated and the residue treated with KHSO<sub>4</sub> 5% giving a yellow solid which was filtered, washed with NaHCO<sub>3</sub> 5%, water and dried.

- 1.05 g of compound were obtained, MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 919; HPLC (conditions of Example 23): Rt = 20.36

c) H-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-COCH[COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]CH<sub>2</sub>COOH

- Boc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-COCH[COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]CH<sub>2</sub>COO-tBu (1.05 g) was added in small portions in anhydrous trifluoroacetic acid (20 ml) at 0°C and the mixture was kept under stirring for 30 minutes, dried and the residue treated with ethyleter; the formed solid was filtered, washed with ethyleter and dried, 850 mg of product were obtained.

MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 763; HPLC (conditions of example 23): Rt = 10.6

- d) Synthesis of cyclo{-Suc[1-COO(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}



The H-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-COCH[COO(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]CH<sub>2</sub>COOH (100 mg) was dissolved in DMF (5 ml) and to the mixture PyBOP (80 mg) and Et<sub>3</sub>N (54 µl) were added stirring for 3 h.

The reaction mixture was dried and the residue dissolved in ethylacetate, the organic phase was washed with KHSO<sub>4</sub> 5%, brine, NaHCO<sub>3</sub> 5% and brine, dried and concentrated. 90 mg of epimeric mixture was obtained, the epimers were separated by HPLC giving:

30 mg of liophylized solid which in HPLC (conditions of example 23) shows an Rt = 15.2. MS(ES+):[MH+]= 745.

1H-NMR (DMSO): δ 2.54-2.81 (m, 7H), 3.08-3.17 (m, 1H), 3.34-3.39 (m, 1H), 3.77-3.84 (m, 1H), 4.00-4.10 (m, 3H), 4.37-4.43 (m, 1H), 5.31 (s, 2H), 6.60 (d, 1H), 6.93-7.42 (m, 16H), 7.62 (d, 2H), 8.24 (d, 2H), 8.60 (d, 1H), 8.66-8.72 (m, 1H), 10.81 (s, 1H) and

7 mg of liophylized solid which in HPLC (conditions of example 23) shows an Rt = 15.7. MS(ES+): [MH+]= 745.

#### Example 29

Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]} (compound of formula I, wherein h = 1, g = 0, R<sub>4</sub> = -COOH and the other substituents are as defined in Example 1) [epimer which in HPLC (conditions of Example 23) shows an Rt = 10.7]

The cyclo{-Suc(1-COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>))-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]} which in HPLC (same conditions as in example 23) shows an Rt = 15.2 (50 mg) was suspended in a mixture water/isopropanol:1/1 (6 ml) containing K<sub>2</sub>CO<sub>3</sub> (19 mg) and was kept under stirring for 24 h. The solvent was evaporated and the residue was diluted with water and the solution washed with ethylacetate, by adding HCl 1N separated a solid which was extracted with ethylacetate; the organic phase was washed with brine and dried. By evaporating the solvent mg 35 of a solid residue were obtained.

MS(ES+):[MH+]=610. HPLC (conditions of Example 23): Rt = 10.7

#### Example 30

Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]} (compound of formula I, wherein h = 1, g = 0, R<sub>4</sub> = -COOH and the other substituents are as

defined in Example 1) [epimer which in HPLC (conditions as in Example 23) shows an  $R_t = 11.1$ ]

The cyclo{-Suc(1-COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>))-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]} having  $R_t = 15.7$  was hydrolyzed as described in Example 29.

5 MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 610; HPLC (same conditions of Example 23):  $R_t = 11.1$

As described in Example 28 the following compounds were obtained :

Example 31

Cyclo{-Suc(1-OH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which  $h = 1$ ;  $g = 0$ ;  $R_4 = -OH$ , and the other  
10 substituents are as defined in Example 1), MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 582.

Example 32

Cyclo{-Suc(2-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}

(compound of formula I, in which  $R_4 = -COOH$ , and the other substituents are  
as defined in Example 1) MS(ES<sup>+</sup>):[MH<sup>+</sup>] = 610.

15 Example 33

Cyclo{-Suc(2-OH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]} (compound of  
formula I wherein:  $h = 0$ ,  $g = 1$ ,  $R_4 = OH$  and the other substituents are as  
defined in example 1) MS(ES<sup>+</sup>): [MH<sup>+</sup>] = 582.

The compounds of Examples 23, 24, 25, 26, 27, 29, 30 and 32 can be  
20 derivatized as described hereinafter.

Example 34

Cyclo{-Suc[1(S)-(2H-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-  
C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}.TFA (compound of formula I wherein  $h = 1$ ,  $g = 0$ ,  $R_4 = -(2H$ -  
tetrazolyl-5-ylmethyl)amino and the other substituents are as defined in  
25 example 1 while the carbon atom C- $R_4$  has configuration S)

a) Synthesis of 5-iodomethyl-1-trityl-1H-tetrazole

To a suspension of 5-chloromethyl-1H-tetrazole (6.0 g) in chloroform (100 ml)  
trityl-chloride (14.2 g) was added at 0°C under nitrogen, and the mixture was  
stirred up to total solubilization, thereafter a solution of Et<sub>3</sub>N (7.0 ml) in  
30 chloroform (50 ml) was added at 5°C and the temperature was left raising up to  
room temperature, the mixture was kept resting for 24 h.

The mixture was treated with ethylacetate (200 ml) and left resting for 6h, the separated solid was filtered away and to the solution acetone (70 ml) was added, the precipitated solid was collected by filtration and dried giving 9.5 g of 5-chloromethyl-1-trityl-1H-tetrazole which was solubilized in acetone (200 ml) at 60°C. Sodium iodide (5.6 g) was added to the solution which was refluxed for 6 h, by cooling precipitated a compound which was filtered, washed with water and dried giving 5.2 g of a white solid.

TLC: R.f. 0.55 (AcOEt/Cyclohexane : 1/3)

b) Synthesis of cyclo{-Suc[1(S)-(2-trityl-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-  
10 [(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}

To 205 mg of cyclo{-Suc[1(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-} (compound of example 23) in anhydrous DMF (5 ml) were added, under stirring 5-iodomethyl-1-trityl-1H-tetrazole (147 mg) and thereafter DIEA (0.06 ml) keeping the temperature at 0°C for 4 h and at room temperature for 3 h. The mixture was treated with water and extracted with ethylacetate, the organic phase was washed with brine and dried. By evaporating the solvent a solid was obtained which was purified by column-chromatography eluting with AcOEt/MeOH = 95/5. 210 mg of product were obtained. MS(ES<sup>+</sup>):[MH<sup>+</sup>]=905; HPLC (conditions of example 23): Rt=15.4.

c) synthesis of cyclo{-Suc[1(S)-(2-HI-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-  
20 [(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}.TFA

To a solution of cyclo{-Suc[1(S)-(2-trityl-tetrazolyl-5-ylmethyl)amino]Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-} (90 mg) in anhydrous DMF (5 ml) a solution of HCl 4M in dioxane (0.6 ml) was added at 0°-5°C, the temperature was brought to room temperature and the mixture was left resting up to end of the reaction (14 h at room temperature and 56 h at 5°C) checking the reaction by HPLC. The solvent was evaporated and the residue treated with AcOEt, the organic phase was washed with brine and dried; evaporating the solvent 30 g of a crude solid are obtained, the solid is purified by HPLC giving 10 g of liophilyzed solid product.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=663; HPLC (conditions of example 23): Rt=9.0

<sup>1</sup>H-NMR (DMSO):  $\delta$  2.62-2.92 (m, 8H), 3.16-3.23 (m, 1H), 3.68-3.74 (m, 1H), 4.00-4.14 (m, 3H), 4.25-4.75 (m, 3H), 6.88-7.42 (m, 17H), 8.30-8.37 (m, 1H), 8.54 (d, 1H), 10.82 (s, 1H).

#### Example 35

- 5 Cyclo{-Suc[1(S)-(morpholin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -(morpholin-4-yl) and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

To a solution of 2.2'-oxydiacetaldehyde (1mmole), excess, in methanole (20 ml)  
10 58 mg of cyclo{-Suc[1(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-} (compound of example 23), 0.2 ml of acetic acid and 12 mg of NaCNBH<sub>3</sub> were added. After 2 h the mixture was diluted with water (10 ml), treated with HCl 1N up to pH 3 and the methanole was evaporated; the solution was treated with NaHCO<sub>3</sub> 5% and the formed solid was extracted with ethylacetate. The organic  
15 phase, after washing with brine and anhydrification, was evaporated giving 58 mg of a solid which was purified by HPLC giving 10 mg of liophylized solid trifluoroacetate.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=651; TLC: R.f.0.20 (CHCl<sub>3</sub>/MeOH:9/1)

<sup>1</sup>H-NMR (DMSO):  $\delta$  2.62-3.00 (m, 8H), 3.27-3.87 (m, 10H), 4.07-4.15 (m, 3H),  
20 4.32-4.38 (m, 1H), 6.62 (d, 1H), 6.94-7.41 (m, 16H), 8.49 -8.64(m, 2H), 10.84 (s, 1H).

Via a similar reductive amination reaction, as described in example 35, the following compounds were obtained:

#### Example 36

- 25 Cyclo{-Suc[1(S)-N(CH<sub>3</sub>)<sub>2</sub>]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -N(CH<sub>3</sub>)<sub>2</sub> and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The synthesis was performed starting from the compound of example 23 using  
30 paraformaldehyde. MS(ES<sup>+</sup>):[MH<sup>+</sup>]=609.

#### Example 37

Cyclo{-Suc[1(S)-(piperidin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -(piperidin-4-yl) and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

- 5 The synthesis was performed starting from the compound of example 23 using glutaraldehyde. MS(ES+):[MH+]=649.

Example 38

- Cyclo{-Suc[1(S)-(N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub> and  
10 the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The synthesis was performed starting from the compound of example 23 using glycolaldehyde. MS(ES+):[MH+]=669.

Example 39

- 15 Cyclo{-Suc[1(S)-(NHCH<sub>2</sub>CH(OH)CH<sub>2</sub>OH)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -NHCH<sub>2</sub>CH(OH)CH<sub>2</sub>OH and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

- The synthesis was performed starting from the compound of example 24 using  
20 D-glyceraldehyde. MS(ES+):[MH+]=655.

Example 40

- Cyclo{-Suc[1(S)-(3-carboxypropanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}. (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -(3-carboxypropanoyl)amino and the other substituents are as defined in example  
25 1 while the carbon atom C-R<sub>4</sub> has configuration S)

- To a solution of the compound of Example 23 (100 mg) in anhydrous DMF (2 ml) succinic anhydride (30 mg) and dimethylamino-pyridine (10 mg) were added and the solution was stirred for 16 h; the solvent was evaporated giving a solid which was solubilized in ethylacetate, washed with citric acid 10%, brine and  
30 dried. By evaporating the solvent a solid compound was collected (90 mg), which purified by HPLC gave 60 mg of a lyophilized solid.

MS(ES+):[MH+]=681; HPLC (conditions as in example 23): Rt=10.8

<sup>1</sup>H-NMR (DMSO):  $\delta$  2.35-2.82 (m, 12H), 3.25-3.28 (m, 1H), 3.66-3.73 (m, 1H), 3.98-4.12 (m, 2H), 4.33-4.38 (m, 1H), 4.67-4.73 (m, 1H), 6.80 (d, 1H), 6.96-7.39 (m, 16H), 8.16-8.23 (m, 2H), 8.51 (d, 1H), 10.89 (s, 1H).

#### Example 41

- 5 Cyclo{-Suc[1(S)-[3-N'-( $\beta$ -D-glucopiranos-1-yl)-carboxamidopropanoyl]amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -[3-N'-( $\beta$ -D-glucopiranos-1-yl)carboxyamidopropanoyl]amine] and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

- 10 The compound of example 40 (90 mg) was dissolved in anhydrous DMF (10 ml) under stirring and in nitrogen atmosphere, to the mixture 55 mg HBT, 25 mg EDC and 24 mg  $\beta$ -D-glucopiranosylamine were added.

- The mixture was left stirring overnight and after evaporation of the solvent the resulting oil was treated with citric acid 10% giving a solid which was filtered,  
15 washed with water and dried. The 80 mg obtained were purified by HPLC giving 40 mg of a liophylized solid.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]= 842; HPLC (conditions of example 23): Rt= 8.2

- <sup>1</sup>H-NMR (DMSO):  $\delta$  2.31-2.81 (m, 12H), 3.00-3.10 (m, 2H), 3.13-3.65 (m, 5H), 3.66-3.75 (m, 1H), 3.97-4.12 (m, 2H), 4.29-4.36 (m, 1H), 4.65-4.75 (m, 2H), 6.78  
20 (d, 1H), 6.95-7.40 (m, 16H), 8.19-8.27 (m, 2H), 8.35 (d, 1H), 8.51 (d, 1H), 10.82 (s, 1H).

#### Example 42

- Cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -  
25 (carboxymethyl)amino and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

a) Synthesis of cyclo{-Suc[1(S)-[(ter-butoxycarbonylmethyl)-amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}

- To a solution of the compound of example 23 (130 mg) in anhydrous DMF (3  
30 ml) DIEA (0.04 ml) and ter-butyle (0.04 ml) bromoacetate were added, the solution was stirred for 2 h and thereafter the mixture was poured in KHSO<sub>4</sub>

5%. The formed solid was filtered, washed with  $\text{NaHCO}_3$ , water and dried. 100 mg of product were obtained.

HPLC (conditions of Example 23):  $R_t = 11.3$

b) Synthesis of cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}] .TFA

The above collected solid (90 mg) was suspended in  $\text{CH}_2\text{Cl}_2$  (5 ml) and TFA (5 ml) was added under stirring at 0°C, the mixture was stirred for 1 h at room temperature. The solution was concentrated and the obtained residue was purified by HPLC giving 40 mg of a liophylized solid.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=639; HPLC (conditions of Example 23):  $R_t=9.4$ .

#### Example 43

Cyclo{-Suc[1(S)-[N'-(β-D-glucopiranos-1-yl)-carboxyamidomethyl]amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}] TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -[N'-(β-D-glucopiranos-1-yl)carboxyamidomethyl]amine] and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The product was obtained starting from the product of Example 42 and β-D-glucopiranosylamine according to the procedure of Example 41.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=800; HPLC (conditions of example 23):  $R_t= 7.6$

#### Example 44

cyclo{-Suc[1(S)-(chiny)amine]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}] (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -(chiny)amine and the other substituents are as defined in example 1. while the carbon atom C-R<sub>4</sub> has configuration S)

Chinic acid (50 mg) was solubilized in anhydrous DMF (10 ml) under stirring and nitrogen atmosphere, HBT (220 mg), EDC (100 mg) and the compound obtained in Example 24 (150 mg) were added. The mixture was left under stirring overnight, thereafter the solvent was evaporated and the residue treated with an aqueous solution of  $\text{KHSO}_4$  5% and extracted with ethylacetate.

The organic phase was washed with brine,  $\text{NaHCO}_3$  5% and again brine, dried and evaporated; the obtained solid (122 mg) was purified on flash

chromatography (SiO<sub>2</sub>) eluting with chloroform/methanol:8/2; 80 mg of the desired compound were obtained.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=755; HPLC (conditions as in example 23) Rt=10.05

#### Example 45

- 5 Cyclo{-Suc[1(S)-(4-aminobutanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -(4-aminobutanoyl)amino and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The product was obtained starting from the product of Example 23 and 4-BOC-aminobutyric acid according to the procedure of Example 44 followed by elimination of the protecting group BOC.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]=666.

#### Example 46

- 15 Cyclo{-Suc[1(S)-[(1,4')bipiperidin-1-yl]acetamido]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -[(1,4')bipiperidin-1-yl]acetamido and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The product was obtained starting from the product of Example 23 and [(1,4')bipiperidin-1-yl]acetic acid according to the procedure of Example 44.

- 20 MS(ES<sup>+</sup>):[MH<sup>+</sup>]=789.

#### Example 47

- 25 Cyclo{-Suc[1-N-(β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = N-(β-D-glucopiranos-1-yl)carboxamide and the other substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The product was obtained starting from the product of Example 29 and β-D-glucopiranosylamine according to the procedure of Example 44.

MS(ES<sup>+</sup>):[MH<sup>+</sup>]= 771.

#### Example 48

- 30 Cyclo{-Suc[1(S)-[N'-(2-N-acetyl-β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} (compound of formula I wherein h = 1, g = 0, R<sub>4</sub> = -N'-(2-N-acetyl-β-D-glucopiranos-1-yl)carboxamide and the other



substituents are as defined in example 1 while the carbon atom C-R<sub>4</sub> has configuration S)

The product was obtained starting from the acid of Example 29 and 2-N-acetyl- $\beta$ -D-glucopiranosylamine according to the procedure of Example 44.

5 MS(ES<sup>+</sup>):[MH<sup>+</sup>]=812.

#### Biological activity

The compounds described in the present invention act as antagonists of the NK-2 receptor of tachykinins. The biological activity was evaluated in two *in-vitro* functional tests, using rabbit pulmonary artery (RPA) and hamster trachea  
10 (HT), according to the methods described by C.A. Maggi *et al.*, Br. J. Pharmacol., 1990, 100, 588 and P. D'Orléans-Juste *et al.*, Eur. J. Pharmacol., 1986, 125, 37. The activity of the compounds as human NK-2 receptor antagonists was assessed in a binding test using membranes of Chinese hamster ovary (CHO) cells, transfected with the NK-2 receptor of human ileum  
15 and the radioligand [<sup>125</sup>I]NKA (Amersham, specific activity 2000 Ci/mmol) at a concentration of 100 pM in competition studies. The substances under examination were tested in a concentration range of from 0.01 nM to 10 mM. At the end of incubation (30 minutes at 20°C), the samples were filtered on Whatman GF/B filters and employing the Brandel automatic filtration system.  
20 Radio-activity was determined by means of a gamma counter (Cobra, Canberra Packard).

The data gathered from the functional studies were expressed as pA<sub>2</sub> (O. Arunlakshana and H.O. Schild, Br. J. Pharmacol. Chemother., 1959, 14, 48), and those of the binding studies as pKi (-log Ki calculated using the LIGAND  
25 programme: P.J. Munson *et al.*, Anal. Biochem., 1980, 107, 220).

The compounds of the invention proved active in the tests referred to above, with pA<sub>2</sub> values of between 5 and 9, the more powerful compounds revealing a higher affinity for the human receptor, with pKi of between 8 and 10.

#### List of abbreviations used

30 For the nomenclature and abbreviations of amino acids, reference is made to the recommendations of the IUPAC-IUB Joint Commission on Biochemical

Nomenclature (Eur. J. Biochem., 1984, 138, 9); the amino acids are understood in the S configuration, if not otherwise specified.

The other abbreviations used are the following:

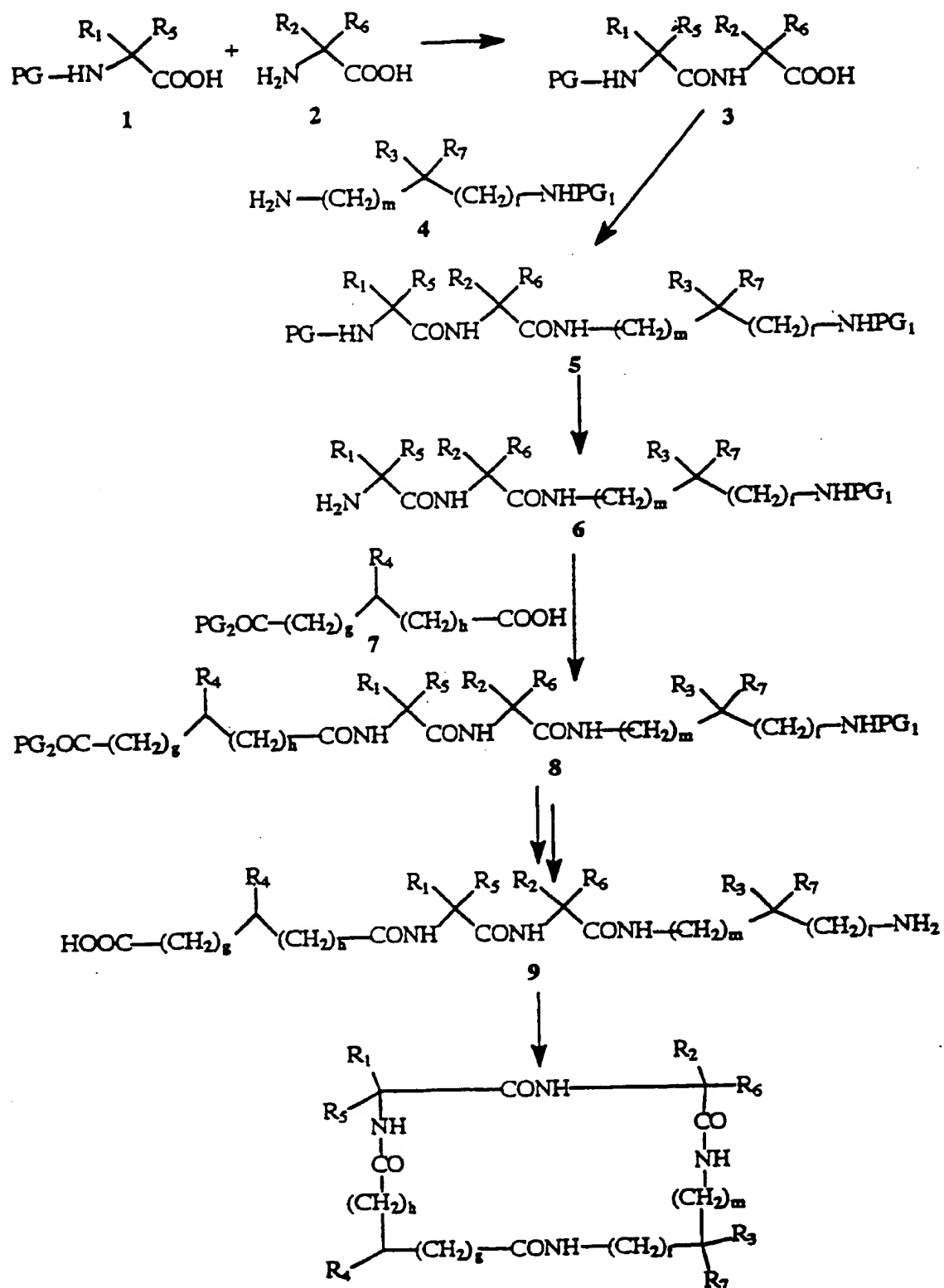
BOC = *tert*-butoxycarbonyl; Z = benzyloxycarbonyl; -Suc- = succinyl; Bzl =  
5 benzyl; PyBOP = (benzotriazol-1-yloxy)*tris*(pyrrolidine) phosphonium  
hexafluorophosphate, DIEA = N,N-diisopropylethylamine; NEt<sub>3</sub> = triethylamine;  
DMF = N,N-dimethylformamide; NKA = neurochinine A; TFA = trifluoro-acetic  
acid; HBT = 1-hydroxybenzotriazole; EDC = N-(3-dimethylaminopropyl)-N'-  
ethylcarbodiimide hydrochloride.

10 The numeration of the substituents on the succinic-group is as follows:

-Suc(1-NH<sub>2</sub>)- = -CO-CH(NH<sub>2</sub>)-CH<sub>2</sub>-CO-

-Suc(2-NH<sub>2</sub>)- = -CO-CH<sub>2</sub>-CH(NH<sub>2</sub>)-CO-

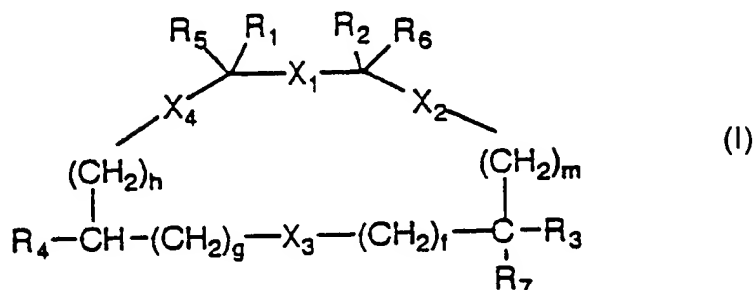
## Scheme



where PG, PG1 and PG2 are protecting groups commonly used in the synthesis of peptides.

## CLAIMS

1. Monocyclic compounds having the general formula (I):



in which:

$X_1, X_2, X_3, X_4$ , which may be the same or different from one another, represent a group chosen from among -CONR-, -NRCO-, -OCO-, -COO-, -CH<sub>2</sub>NR-, -NR-CH<sub>2</sub>-, CH<sub>2</sub>-CH<sub>2</sub>, where R is H or a C<sub>1-3</sub> alkyl or benzyl;

f, g, h, m, which may be the same or different from one another, represent a number chosen from among 0, 1 or 2;

$R_1$  and  $R_2$ , which may be the same or different from one another, represent a -(CH<sub>2</sub>)<sub>r</sub>-Ar group, where r = 0, 1, 2 and where Ar is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar group being possibly substituted with a maximum of 2 residues chosen from among C<sub>1-3</sub> alkyl or halo-alkyl, C<sub>1-3</sub> alkoxy, C<sub>2-4</sub> amino-alkoxy, halogen, OH, NH<sub>2</sub>, NR<sub>13</sub>R<sub>14</sub> where R<sub>13</sub> and R<sub>14</sub>, which may be the same or different from one another, represent hydrogen or C<sub>1-3</sub> alkyl;

$R_3$  represents a group chosen from among:

- hydrogen

- linear or branched alkyl having the formula C<sub>n</sub>H<sub>2n+1</sub>, with n = 1-5, cyclo-alkyl or alkylcyclo-alkyl groups having the formula C<sub>n</sub>H<sub>2n-1</sub> with n = 5-9

-(CH<sub>2</sub>)<sub>r</sub>-Ar<sub>1</sub>, where r = 0, 1, 2 and where Ar<sub>1</sub> is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar<sub>1</sub> group being possibly substituted with a maximum of 2 residues chosen from among C<sub>1-3</sub> alkyl or halo-alkyl, C<sub>1-3</sub> alkoxy or amino-alkoxy, halogen, OH, NH<sub>2</sub>, NR<sub>13</sub>R<sub>14</sub>, where R<sub>13</sub> and R<sub>14</sub>, which may be the same or different from one another, represent hydrogen or C<sub>1-3</sub> alkyl;

32  $R_4$  represents a group chosen from among:

33 - hydrogen or  $C_{1-6}$  alkyl

34 - L-Q, where L is a chemical bond or a linear or branched  $C_{1-6}$  alkyl residue and

35 Q is a group chosen from among:

36 i) H, OH,  $OR_9$ ,  $NH_2$ ,  $NR_9R_{10}$ , guanidine, sulphate, phosphonate, phosphate,  
37 where  $R_9$  and  $R_{10}$ , which may be the same or different from one another,  
38 represent a hydrogen,  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxyalkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$   
39 alkyl- $CONHR_{12}$ ,  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or wherein  $R_9R_{10}$  joined  
40 together form with the N-atom a saturated 4-6 membered heterocycle possibly  
41 containing a further heteroatom chosen in the group consisting of N, O, S and  
42 wherein  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly protected with one or  
43 more  $C_{1-3}$ -acyl groups or substituted with amino-groups or  $C_{1-3}$ acylamino-  
44 groups;

45 ii) COOH, tetrazole,  $SO_2NH_2$ ,  $SO_2NHCOOR_8$ ,  $CONHR_8$ ,  $NHCOR_8$ , where  $R_8$   
46 represents a linear or cyclic  $C_{1-6}$  alkyl chain containing one or more polar groups  
47 chosen from among the group: OH,  $NH_2$ ,  $NR_{15}R_{16}$ , COOH,  $CONHR_{12}$ ,  $PO_3H$ ,  
48  $SO_3H$ ,  $OR_{11}$  and where  $R_{15}$  and  $R_{16}$ , which may be the same or different from  
49 one another, represent a hydrogen or  $C_{1-3}$  alkyl group, and where  $R_{11}$  is a  $C_{1-3}$   
50 alkyl or  $C_{2-4}$  amino-alkyl chain,  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly  
51 protected with one or more  $C_{1-3}$ acyl groups or substituted with amino-groups or  
52  $C_{1-3}$ acylamino-groups or  $R_{15}R_{16}$  joined together form with the N-atom a  
53 saturated 4-6 membered heterocycle possibly substituted with  $C_{1-3}$ alkyl-groups  
54 or with saturated 4-6 membered heterocycle-groups containing at least an N-  
55 atom;

56 iii)  $COOR_{17}$ ,  $CONHR_{12}$ ,  $OR_{12}$  where  $R_{12}$  is a mono-, di- or tri-glycoside group  
57 possibly protected with one or more  $C_{1-3}$  acyl groups or substituted with amine  
58 or  $C_{1-3}$  acylamine groups and  $R_{17}$  is a group  $R_{12}$  as above defined or a group  
59  $C_{1-3}$ alkyl,  $C_{1-3}$ alkylphenyl, wherein the phenyl-group can be substituted with a  
60 group OH,  $NO_2$ ,  $NH_2$ , CN,  $CH_3$ , Cl, Br;

61  $R_5$ ,  $R_6$ ,  $R_7$ , which may be the same or different from one another, represent a  
62 hydrogen or  $C_{1-3}$  alkyl group; their pharmaceutically acceptable salts, their  
63 enantiomers and mixture thereof.

- 1 2. Compounds according to Claim 1, in which:  
2 f, g, h, m, which may be the same or different from one another, may be 0 or 1;  
3  $R_1$  and  $R_2$ , which may be the same or different from one another, represent the  
4 side chain of a natural amino acid chosen from among tryptophan, phenyl  
5 alanine, tyrosine, histidine or the side chain of a non-natural amino acid chosen  
6 in the group:  
7 tryptophan and phenyl alanine, either mono- or di-substituted with residues  
8 chosen from among  $C_{1-3}$  alkyl or halo-alkyl,  $C_{1-3}$  alkoxy or amino-alkoxy,  
9 halogen, OH,  $NH_2$ ,  $NR_{13}R_{14}$ , where  $R_{13}$  and  $R_{14}$ , which may be the same or  
10 different from one another, represent a hydrogen or  $C_{1-3}$  alkyl group;  
11  $R_3$  represents a group chosen from among:  
12 - linear or branched alkyl having the formula  $C_nH_{2n+1}$ , with  $n = 1-5$  (chosen in the  
13 group consisting of methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl) cycloalkyl or  
14 alkylcycloalkyl of formula  $C_nH_{2n-1}$  with  $n = 5-9$  (chosen in the group consisting of  
15 cyclopentyl, cyclohexyl, methylcyclohexyl)  
16 -  $(CH_2)_r-Ar_1$ , where  $r = 1$  or  $2$  and where  $Ar_1$  is an aromatic group chosen in the  
17 group consisting of:  $\alpha$ -naphthyl,  $\beta$ -naphthyl, phenyl, indole, the said  $Ar_1$  group  
18 being possibly substituted with a maximum of 2 residues chosen in the group  
19 consisting of:  $C_{1-3}$  alkyl,  $CF_3$ ,  $C_{1-3}$  alkoxy, Cl, F, OH,  $NH_2$ ;  
20  $R_4$  represents an L-Q group where:  
21 L is a chemical bond or  $CH_2$ , and  
22 Q is a group chosen from among:  
23 - OH,  $NH_2$ ,  $NR_9R_{10}$ ,  $OR_{11}$ , and where  $R_9$  and  $R_{10}$ , which may be the same or  
24 different from one another, represent a hydrogen or  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxy  
25 alkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$ alkyl-CONHR<sub>12</sub> (wherein  $R_{12}$  is a monoglycosidic  
26 group derived from D or L pentoses or hexoses (chosen in the group consisting  
27 of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine  
28 and their N-acetylated derivatives)),  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or  
29 wherein  $R_9R_{10}$  are joined together to form with the N atom a morpholine or a  
30 piperidine ring and where  $R_{11}$  is a  $C_{1-3}$  alkyl chain, or a  $C_{2-4}$  amino-alkyl chain;

- 31 -  $\text{NHCOR}_8$  wherein  $R_8$  is a cyclohexane containing from 2 to 4 OH groups, a  $\text{C}_{1-6}$   
 32 alkylchain containing a polar group (chosen in the group consisting of  $\text{NH}_2$ ,  
 33  $\text{COOH}$ ,  $\text{CONHR}_{12}$  (wherein  $R_{12}$  is as hereabove define) or [1,4']bipiperidine)  
 34 -  $\text{COOH}$ ,  $\text{COOR}_{17}$  or  $\text{CONHR}_{12}$ , wherein  $R_{12}$  is as hereabove defined and  $R_{17}$  is  
 35 as  $R_{12}$  or a group 4-nitrobenzyl.  
 36 -  $R_5$ ,  $R_6$ ,  $R_7$  are H.

37 in which the carbon atom that carries the substituents  $R_3$  and  $R_7$  has  
 38 configuration R.

1 3. Compounds according to Claim 2, as specified below:

- 2 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 3 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 4 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>11</sub>)-CH<sub>2</sub>-NH-]}
- 5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>(4-OCH<sub>3</sub>))-CH<sub>2</sub>-NH-]}
- 6 Cyclo{-Suc-Trp(5F)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 7 Cyclo{-Suc-Trp(Me)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 8 Cyclo{-Suc-Phe(3,4-Cl)-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 9 Cyclo{-Suc-Trp-Phe(3,4-Cl)-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 10 Cyclo{-Suc-Trp-Tyr-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 11 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>-3,4-diCl)-CH<sub>2</sub>-NH-]}
- 12 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-4-OH)-CH<sub>2</sub>-NH-]}
- 13 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}
- 14 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-2-naphthyl)-CH<sub>2</sub>-NH-]}
- 15 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-indol-3-yl)-CH<sub>2</sub>-NH-]}
- 16 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-5-F-indol-3-yl)-CH<sub>2</sub>-NH-]}
- 17 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-3-F)-CH<sub>2</sub>-NH-]}
- 18 Cyclo{-Suc-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>-3,4-diF-CH<sub>2</sub>-NH)-]}
- 19 Cyclo{-Suc-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-CF<sub>3</sub>-CH<sub>2</sub>-NH)-]}
- 20 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-NH-]}
- 21 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH<sub>2</sub>-CH(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)-NH-]}
- 22 Cyclo{-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH-]}-(CH<sub>2</sub>)<sub>3</sub>CO-}
- 23 Cyclo{-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-N(CH<sub>3</sub>)]-(CH<sub>2</sub>)<sub>3</sub>CO-}
- 24 Cyclo{-Suc[1(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-]}

- 25 Cyclo{-Suc[1(R)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}
- 26 Cyclo{-Suc[2(S)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}
- 27 Cyclo{-Suc[2(R)-NH<sub>2</sub>]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}
- 28 Cyclo{-Suc[1(S)-NH(CH<sub>3</sub>)]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}
- 29 Cyclo{-Suc[1-COO(CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-4-NO<sub>2</sub>)]-Trp-Phe-[(R)NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}
- 30 Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 31 Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 32 Cyclo{-Suc(1-OH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 33 Cyclo{-Suc(2-COOH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 34 Cyclo{-Suc(2-OH)-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 35 Cyclo{-Suc[1(S)-(2H-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-
- 36 C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.TFA
- 37 Cyclo{-Suc[1(S)-(morpholin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-
- 38 }.TFA
- 39 Cyclo{-Suc[1(S)-N(CH<sub>3</sub>)<sub>2</sub>]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}.TFA
- 40 Cyclo{-Suc[1(S)-(piperidin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>NH]-}.TFA
- 41 Cyclo{-Suc[1(S)-(N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-
- 42 NH]-}.TFA
- 43 Cyclo{-Suc[1(S)-(N(CH<sub>2</sub>CH(OH)CH<sub>2</sub>OH)]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-
- 44 NH]-}.TFA
- 45 Cyclo{-Suc[1(S)-(3-carboxypropanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-
- 46 CH<sub>2</sub>-NH]-}.
- 47 Cyclo{-Suc[1(S)-[3-N'-(β-D-glucopiranos-1-yl)-carboxamidopropanoyl]amino]-
- 48 Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 49 Cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-
- 50 NH]-} TFA
- 51 Cyclo{-Suc[1(S)-[N'-(β-D-glucopiranos-1-yl)-carboxyamidomethyl]amino]-Trp-
- 52 Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} TFA
- 53 Cyclo{-Suc[1(S)-(chiny)amine]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}
- 54 Cyclo{-Suc[1(S)-(4-aminobutanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-
- 55 NH]-} TFA

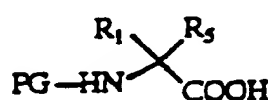


56 Cyclo{-Suc[1(S)-[(1,4')bipiperidin-1-yl]acetamido]-Trp-Phe-[(R)-NH-CH(CH<sub>2</sub>-  
 57 C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-} TFA

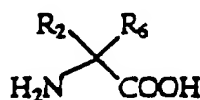
58 Cyclo{-Suc[1-N-(β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-  
 59 CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}

60 Cyclo{-Suc[1(S)-[N'-(2-N-acetyl-β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-  
 61 [(R)-NH-CH(CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-NH]-}.

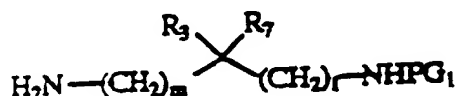
- 1 4. Process for the synthesis of a compound of general formula (I), where X<sub>1</sub>, X<sub>2</sub>,  
 2 X<sub>3</sub>, X<sub>4</sub> are CONH and the other substituents are as defined in Claim 1, where:  
 3 a) the suitably protected amino acids (1), (2) and (4)



(1)

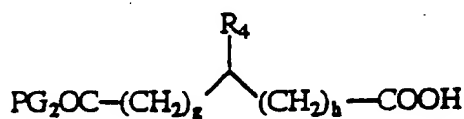


(2)



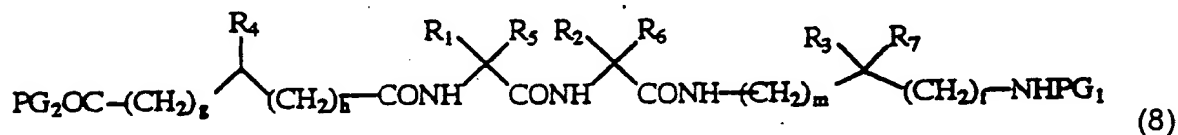
(4)

9 are made to react, as shown in the diagram, with the derivative of the protected  
 10 succinic acid (7)

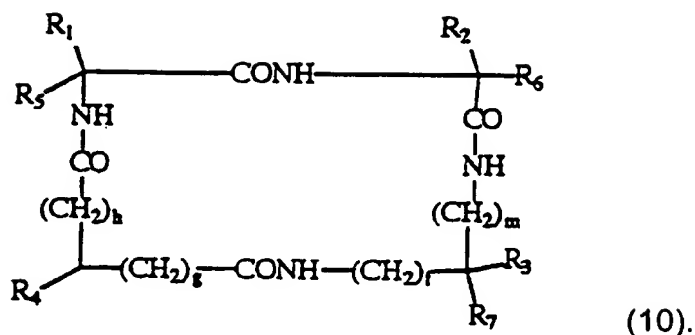


(7)

16 thus obtaining the linear compound (8)



21 b) the linear compound 8, is deprotected and cyclicized to yield the final  
 22 monocyclic compound (10)



5. Pharmaceutical compositions containing as active principle the compounds of general formula (I) according to Claim 1 in combination with pharmaceutically acceptable carriers or excipients.

6. Pharmaceutical compositions according to Claim 5, to be used as tachykinin antagonists.

7. Pharmaceutical compositions according to Claim 6, to be used as antagonists of the human NK-2 receptor.

8. Pharmaceutical compositions according to Claim 7, to be used in the treatment of the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, and kidney infections and colics.

9. Pharmaceutical compositions according to Claim 7, to be used as anxiolytics.

10. Use of a compound according to Claim 1 as tachykinin antagonist.

11. Use of a compound according to Claim 1 as NK-2 antagonist.

12. Use of a compound according to Claim 1 in the treatment of the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, and kidney infections and colics.

13. Use of a composition according to Claim 1 as an NK-2 antagonist for the treatment of anxiety syndromes.

1 14. Method for the treatment of the bronchospastic and inflammatory  
2 component of asthma, coughing, pulmonary irritation, intestinal spasms,  
3 spasms of the biliary tract, local spasms of the bladder and of the ureter during  
4 cystitis, and kidney infections and colics, in which quantities of between 0.02  
5 and 10 mg/kg of body weight of active principle consisting of products of  
6 formula (I), according to Claim 1, are administered to the patient.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00599

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C07K5/065 C07K7/54

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 703 034 A (FREIDINGER ROGER ET AL) 27 October 1987 see column 11; claim 1; table IV	1,2
X	KITABATAKE K. ET AL.: "GUSHING- INDUCING PEPTIDES IN BEER PRODUCED BY PENICILLUM CHYRSOGENUM" PEPT.CHEM, vol. 17, 1980, TOKYO, pages 7-12, XP002073620 see table 3	1,2
Y	WO 96 28467 A (MENARINI FARMA IND ; ARCAMONE FEDERICO (IT); MAGGI CARLO ALBERTO (I) 19 September 1996 see claim 1	1-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

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14/08/1998

Name and mailing address of the ISA  
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Fax: (+31-70) 340-3016

Authorized officer

Deffner, C-A

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00599

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>EP 0 333 174 A (FUJISAWA PHARMACEUTICAL CO) 20 September 1989 see claim 1</p> <p>-----</p>	1-14

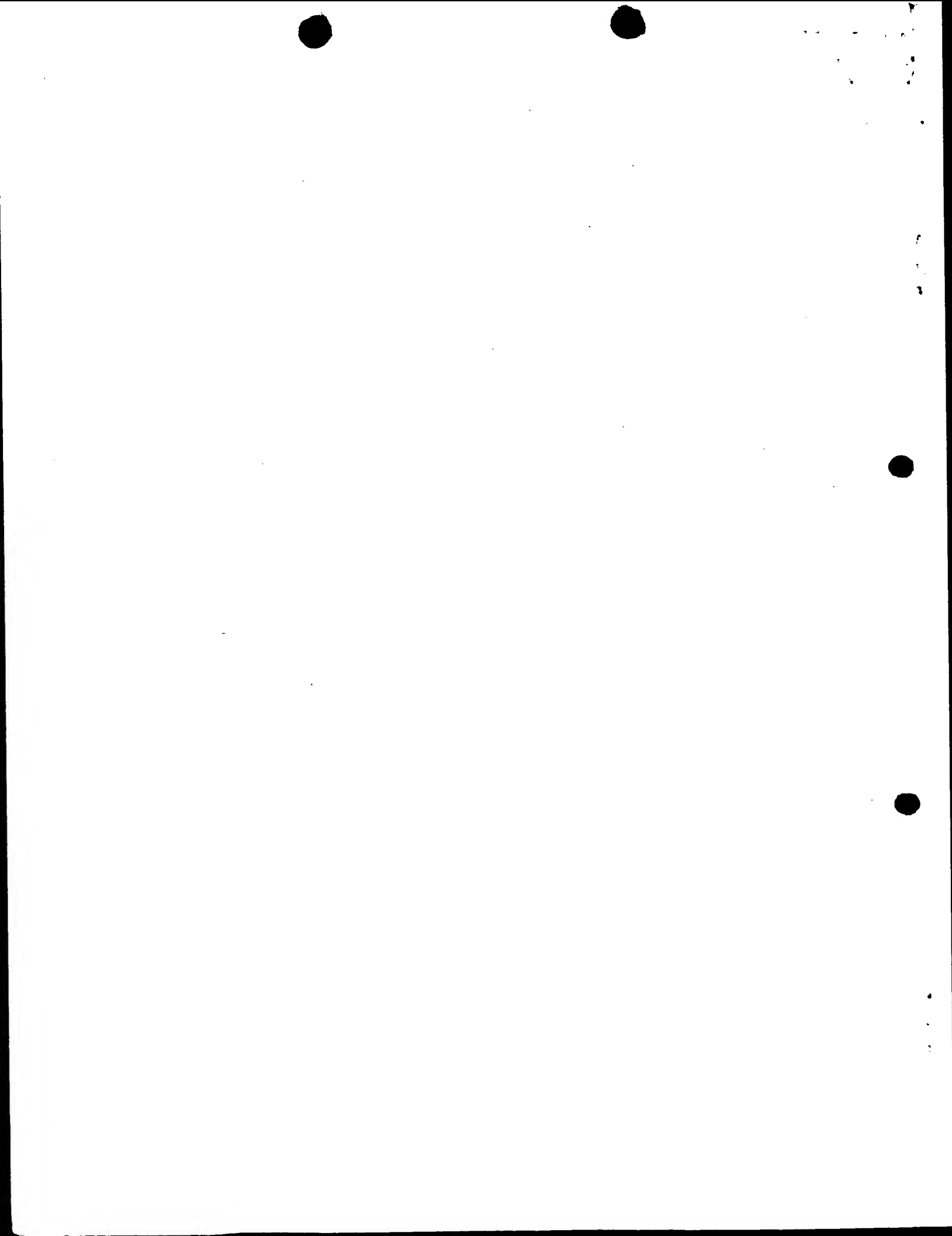
# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/00599

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4703034	A	27-10-1987	NONE	
WO 9628467	A	19-09-1996	IT FI950044 A	13-09-1996
			AU 5105996 A	02-10-1996
			BR 9607348 A	30-12-1997
			CA 2215372 A	19-09-1996
			CZ 9702862 A	18-02-1998
			EP 0815126 A	07-01-1998
			HR 960117 A	31-08-1997
			NO 974057 A	07-11-1997
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			SK 121297 A	04-02-1998
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			AU 3132489 A	21-09-1989
			CA 1329444 A	10-05-1994
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			DE 68926403 T	17-10-1996
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			FI 891176 A	17-09-1989
			JP 1287095 A	17-11-1989
			US 5187156 A	16-02-1993





R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, which may be the same or different from one another, represent a hydrogen or C<sub>1-3</sub> alkyl group.

Also included in the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions  
5 containing them.

In view of the presence of chiral centres in the compounds of formula (I), also the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

#### State of the art

10 The NK-2 receptor of tachykinins is widely expressed in the peripheral nervous system of mammals. One of the various effects produced by the selective stimulation of the NK-2 receptor is the contraction of smooth muscle. Hence antagonists of the NK-2 receptor may be considered agents capable of controlling excessive contraction of smooth muscle in any pathological  
15 condition in which the release of tachykinins concurs in the genesis of the corresponding disorder.

In particular, the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, kidney infections  
20 and colics may be considered conditions in which the administration of NK-2 antagonists may be effective (E.M. Kudlacz *et al.*, Eur. J. Pharmacol., 1993, 241, 17-25).

In addition, a number of NK-2 antagonists capable of surmounting the haemato-encephalic barrier have shown anxiolytic properties (D.M. Walsh *et al.*, Psychopharmacology, 1995, 121, 186-191).  
25

Cyclic compounds, and in particular cyclic hexapeptides (A.T. McKnight *et al.*, Br. J. Pharmacol., 1991, 104, 355) and bicyclic hexapeptides (V. Pavone *et al.*, WO 93/212227) or cyclic hexapeptideptides (L. Quartara *et al.*, J. Med. Chem., 1994, 37, 3630; S.L. Harbeson *et al.*, Peptides, Chemistry and Biology.  
30 Proceedings of the Twelfth American Peptide Symposium, 1992, 124) are known in the literature for their antagonistic activity towards the NK-2 receptor of tachykinins.



32  $R_4$  represents a group chosen from among:

33 - hydrogen or  $C_{1-6}$  alkyl

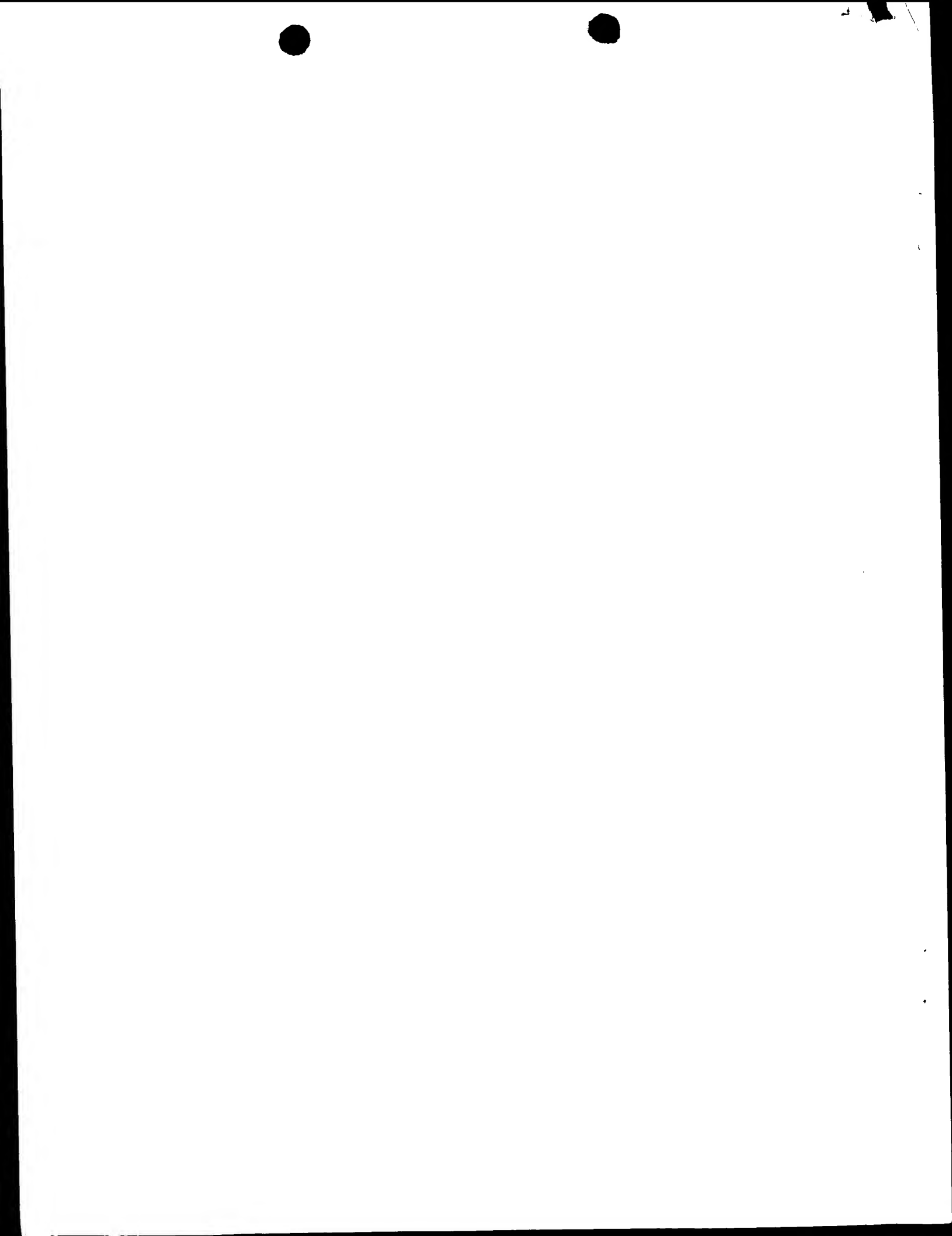
34 - L-Q, where L is a chemical bond or a linear or branched  $C_{1-6}$  alkyl residue and  
35 Q is a group chosen from among:

36 i) H, OH,  $OR_9$ ,  $NH_2$ ,  $NR_9R_{10}$ , guanidine, sulphate, phosphonate, phosphate,  
37 where  $R_9$  and  $R_{10}$ , which may be the same or different from one another,  
38 represent a hydrogen,  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxyalkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$   
39 alkyl- $CONHR_{12}$ ,  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or wherein  $R_9R_{10}$  joined  
40 together form with the N-atom a saturated 4-6 membered heterocycle possibly  
41 containing a further heteroatom chosen in the group consisting of N, O, S and  
42 wherein  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly protected with one or  
43 more  $C_{1-3}$ -acyl groups or substituted with amino-groups or  $C_{1-3}$ acylamino-  
44 groups;

45 ii) COOH, tetrazole,  $SO_2NH_2$ ,  $SO_2NHCOOR_8$ ,  $CONHR_8$ ,  $NHCOR_8$ , where  $R_8$   
46 represents a linear or cyclic  $C_{1-6}$  alkyl chain containing one or more polar groups  
47 chosen from among the group: OH,  $NH_2$ ,  $NR_{15}R_{16}$ , COOH,  $CONHR_{12}$ ,  $PO_3H$ ,  
48  $SO_3H$ ,  $OR_{11}$  and where  $R_{15}$  and  $R_{16}$ , which may be the same or different from  
49 one another, represent a hydrogen or  $C_{1-3}$  alkyl group, and where  $R_{11}$  is a  $C_{1-3}$   
50 alkyl or  $C_{2-4}$  amino-alkyl chain,  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly  
51 protected with one or more  $C_{1-3}$ acyl groups or substituted with amino-groups or  
52  $C_{1-3}$ acylamino-groups or  $R_{15}R_{16}$  joined together form with the N-atom a  
53 saturated 4-6 membered heterocycle possibly substituted with  $C_{1-3}$ alkyl-groups  
54 or with saturated 4-6 membered heterocycle-groups containing at least an N-  
55 atom;

56 iii)  $COOR_{17}$ ,  $CONHR_{12}$ ,  $OR_{12}$  where  $R_{12}$  is a mono-, di- or tri-glycoside group  
57 possibly protected with one or more  $C_{1-3}$  acyl groups or substituted with amine  
58 or  $C_{1-3}$  acylamine groups and  $R_{17}$  is a group  $R_{12}$  as above defined or a group  
59  $C_{1-3}$ alkyl,  $C_{1-3}$ alkylphenyl, wherein the phenyl-group can be substituted with a  
60 group OH,  $NO_2$ ,  $NH_2$ , CN,  $CH_3$ , Cl, Br;

61  $R_5$ ,  $R_6$ ,  $R_7$ , which may be the same or different from one another, represent a  
62 hydrogen or  $C_{1-3}$  alkyl group; their pharmaceutically acceptable salts, their  
63 enantiomers and mixture thereof.



## INTERNATIONAL SEARCH REPORT

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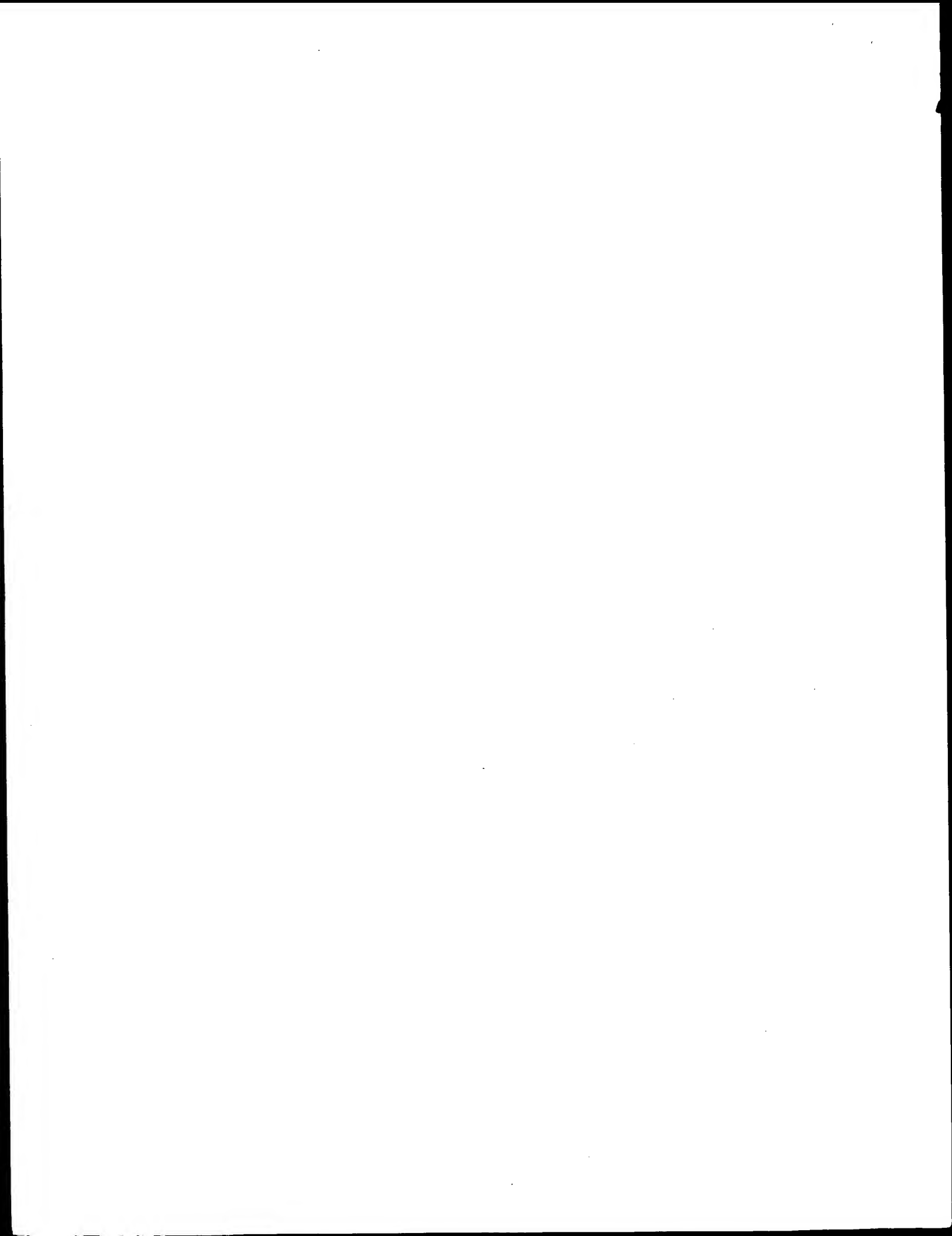
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Fax: (+31-70) 340-3016

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## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets

(11) Publication number:

**0 333 174**  
**A2**

(12)

# EUROPEAN PATENT APPLICATION

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(51) Int. Cl.<sup>4</sup>: C07K 5/00 , A61K 37/02

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30.01.89 GB 8901964

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(54) Peptide compounds, processes for preparation thereof and pharmaceutical composition comprising the same.

(57) A compound of the formula :

$R^1\text{-A-D-Trp}(R^2)\text{-Phe-R}^3$

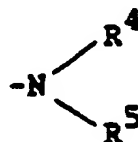
wherein

$R^1$  is hydrogen or an amino protective group,

$R^2$  is hydrogen, an amino protective group, carbamoyl(lower)alkyl, carboxy(lower)alkyl or protected carboxy-(lower)alkyl,

$R^3$  is ar(lower)alkyl,

a group of the formula:



wherein  $R^4$  and  $R^5$  are each hydrogen, aryl or lower alkyl which may have suitable substituent(s), or

$R^4$  and  $R^5$  are linked together to form benzene-condensed lower alkylene, or

a group of the formula :

$\text{-OR}^6$

wherein  $R^6$  is hydrogen, aryl or lower alkyl which may have suitable substituent(s), and

A is a single bond or one or two amino acid(s) residue, provided that when A is one amino acid residue of

-D-Trp-, then  $R^4$  is not hydrogen,

and a pharmaceutically acceptable salt thereof,

processes for its preparation and pharmaceutical compositions comprising them or a pharmaceutically

**EP 0 333 174 A2**

acceptable salt thereof in admixture with pharmaceutically acceptable carriers.

# PEPTIDE COMPOUNDS, PROCESSES FOR PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

The present invention relates to new peptide compounds and pharmaceutically acceptable salts thereof.

More particularly, it relates to new peptide compounds and pharmaceutically acceptable salts thereof which have pharmacological activities such as tachykinin antagonism and the like, to processes for preparation thereof, to pharmaceutical composition comprising the same, and to a method of using the same therapeutically in the treatment and the prevention of asthma and the like.

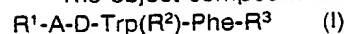
One object of the present invention is to provide new and useful peptide compounds and pharmaceutically acceptable salts thereof which have pharmacological activities such as tachykinin antagonism and the like.

Another object of the present invention is to provide processes for the preparation of said peptide compounds and salts thereof.

A further object of the present invention is to provide a pharmaceutical composition comprising, as an active ingredient, said peptide compounds and pharmaceutically acceptable salts thereof.

Still further object of the present invention is to provide a method for the treatment and the prevention of asthma and the like.

The object compound of the present invention can be represented by the following general formula (I).



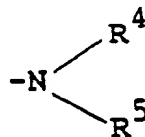
wherein

$R^1$  is hydrogen or an amino protective group,

$R^2$  is hydrogen, an amino protective group, carbamoyl(lower)alkyl, carboxy(lower)alkyl or protected carboxy-(lower)alkyl,

$R^3$  is ar(lower)alkyl,

a group of the formula :



wherein  $R^4$  and  $R^5$  are each hydrogen, aryl or lower alkyl which may have suitable substituent(s), or

$R^4$  and  $R^5$  are linked together to form benzene-condensed lower alkylene, or a group of the formula:

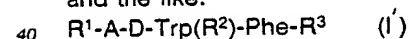


wherein  $R^6$  is hydrogen, aryl or lower alkyl which may have suitable substituent(s), and

A is a single bond or one or two amino acid(s) residue,

provided that when A is one amino acid residue of -D-Trp-, then  $R^4$  is not hydrogen.

Particularly, the compound represented by the following formula (I') is useful as tachykinin antagonist and the like.



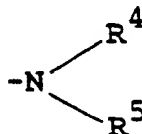
wherein

$R^1$  is hydrogen or an amino protective group,

$R^2$  is hydrogen, an amino protective group, carbamoyl(lower)alkyl, carboxy(lower)alkyl or protected carboxy-(lower)alkyl,

$R^3$  is ar(lower)alkyl,

a group of the formula :



wherein  $R^4$  is hydrogen, aryl or lower alkyl which may have suitable substituent(s), and  $R^5$  is aryl or lower alkyl which may have suitable substituent(s), or

$R^4$  and  $R^5$  are linked together to form benzene-condensed lower alkylene, or

a group of the formula :

5 -OR<sup>6</sup>

wherein  $R^6$  is aryl or lower alkyl which may have suitable substituent(s) and

A is a single bond or one or two amino acid(s) residue.

According to the present invention, the new peptide compounds (I) can be prepared by processes which are illustrated in the following schemes.

### 10 Process 1

H-Phe-R<sup>3</sup>

(III)

or its reactive derivative  
at the amino group  
or a salt thereof

15  $R_a^1$ -A-D-Trp(R<sup>2</sup>)-OH

20 (II)

or its reactive derivative  
at the carboxy group or  
a salt thereof

$R_a^1$ -A-D-Trp(R<sup>2</sup>)-Phe-R<sup>3</sup>

30 (Ia)

or a salt thereof

### Process 2

Elimination of the  
amino protective group

35  $R_a^1$ -A-D-Trp(R<sup>2</sup>)-Phe-R<sup>3</sup>

40 (Ia)

or a salt thereof

H-A-D-Trp(R<sup>2</sup>)-Phe-R<sup>3</sup>

45 (Ib)

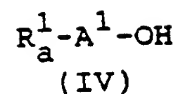
or a salt thereof

50

55

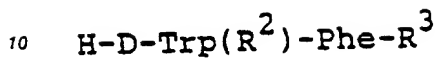
Process 3

5



or its reactive derivative  
at the carboxy group or  
a salt thereof

---

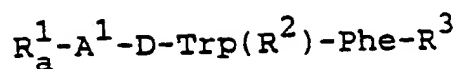


(Ic)

15

or its reactive derivative  
at the amino group or  
a salt thereof

20



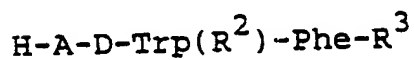
25

(Id)

or a salt thereof

30 Process 4

35



Introduction of the  
amino protective group

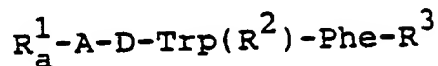
---

(Ib)

40

or its reactive derivative  
at the amino group or  
a salt thereof

45

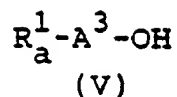


50

(Ia)

or a salt thereof

55

Process 5

or its reactive derivative  
at the carboxy group or  
a salt thereof

---



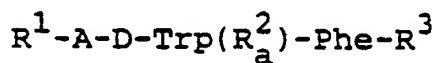
(Ie)

or its reactive derivative  
at the amino group or  
a salt thereof



(If)

or a salt thereof

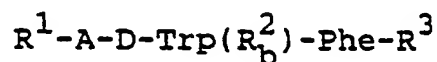
Process 6

Elimination of the  
carboxy protective group

---

(Ig)

or a salt thereof

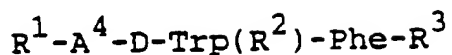


(Ih)

or a salt thereof

Process 7

5

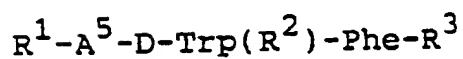


Elimination of the amino,  
hydroxy or carboxy  
protective group

10

(Ii)

or a salt thereof



15

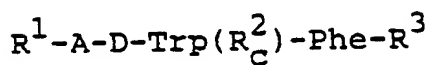
(Ij)

or a salt thereof

20

Process 8

25

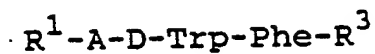


Elimination of the amino  
protective group

30

(Ik)

or a salt thereof



35

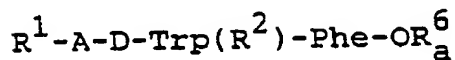
(Il)

or a salt thereof

40

Process 9

45

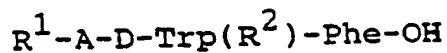


Elimination of  $R_a^6$

50

(Im)

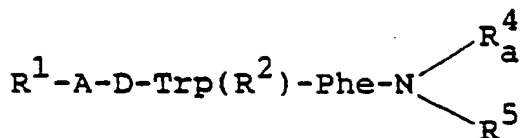
or a salt thereof



55

(In)

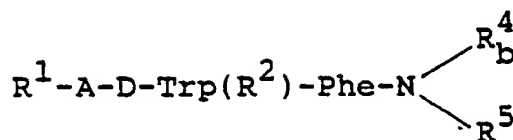
or a salt thereof

Process 10

Elimination of the hydroxy  
protective group

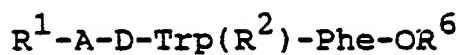
(Io)

or a salt thereof



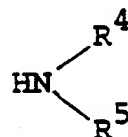
(Ip)

or a salt thereof

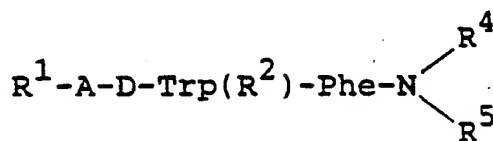
Process 11

(Iq)

or a salt thereof



(VI)



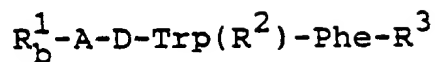
(Ir)

or a salt thereof



Process 12

5



Elimination of the carboxy  
protective group

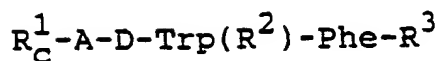
---

10

(Is)

or a salt thereof

15



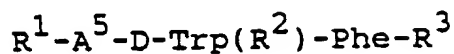
(It)

or a salt thereof

20

Process 13

25



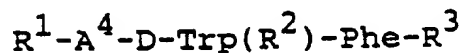
Introduction of the amino,  
hydroxy or carboxy  
protective group

---

(Ij)

or a salt thereof

30

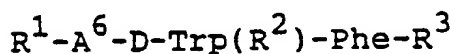


35

(Ii)

or a salt thereof

40

Process 14

Elimination of the amino  
protective group

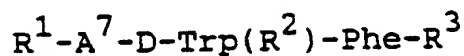
---

45

(Iu)

or a salt thereof

50



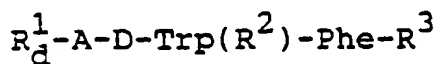
(Iv)

or a salt thereof

55

Process 15

5



Elimination of the amino  
and/or carboxy  
protective group

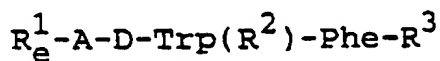
10

(Iw)

or a salt thereof



15

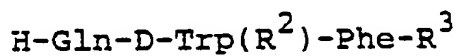


(Ix)

or a salt thereof

Process 16

20



Ring closure

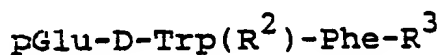
25

(Iy)

or a salt thereof



30



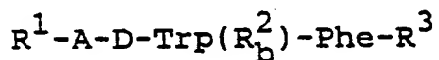
(Iz)

or a salt thereof

35

Process 17

40

 $NH_3$ 

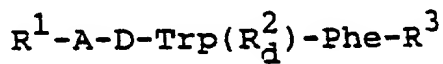
(Ih)

45

or a salt thereof



50



(Izz)

or a salt thereof

wherein

55

 $R^1, R^2, R^3, R^4, R^5, R^6$  and A are each defined above, $R_d^1$  and  $R_b^2$  are each an amino protective group, $R_b^2$  is an amino protective group containing a protected carboxy, $R_b^2$  is an amino protective group containing a carboxy,

$R_d^1$  is an amino protective group containing an amino group which is substituted by an amino protective group and additionally a protected carboxy(lower)alkyl or an ar(lower)alkyl,  
 $R_e^1$  is an amino protective group containing an amino group which is substituted by a carboxy(lower)alkyl or an ar(lower)alkyl,

5  $R_f^2$  is protected carboxy(lower)alkyl,

$R_g^2$  is carboxy(lower)alkyl,

$R_h^2$  is carbamoyl(lower)alkyl,

$R_i^4$  is protected hydroxy(lower)alkyl,

$R_j^4$  is hydroxy(lower)alkyl,

10  $R_k^6$  is lower alkyl which may have suitable substituent(s),

$A^1$  is one or two amino acid(s) residue,

$A^2$  and  $A^3$  are each an amino acid residue,

$A^4$  is one or two amino acid(s) residue containing a protected hydroxy group, a protected amino group, a protected imino group or a protected carboxy group,

15  $A^5$  is one or two amino acid(s) residue containing a hydroxy group, an amino group, an imino group or a carboxy group,

$A^6$  is one or two amino acid(s) residue which is substituted by acyl having protected amino, and

$A^7$  is one or two amino acid(s) residue which is substituted by acyl having amino.

20 As to the starting compounds (II), (III), (IV) and (V) some of them are novel and can be prepared by the procedures described in the Preparation 1 to 22 mentioned later or a conventional manner.

Throughout the present specification, the amino acids, peptides, protective groups, condensing agents, etc. are indicated by the abbreviations according to the IUPAC-IUB (Commission on Biological Nomenclature) which are in common use in the field of art.

25 Moreover, unless otherwise indicated, the amino acids and their residues when shown by such abbreviations are meant to be L-configured compounds and residues, while the D-configured compounds and residues are shown with the prescript of D-.

Suitable pharmaceutically acceptable salts of the object compounds (I) are conventional non-toxic salt and include an acid addition salt such as an organic acid salt (e.g. acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, formate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, hydriodide, sulfate, nitrate, phosphate, etc.), or a salt with an amino acid (e.g. arginine, aspartic acid, glutamic acid, etc.), or a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) and an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, etc.), or the like.

35 In the above and subsequent descriptions of the present specification, suitable examples and illustrations of the various definitions which the present invention include within the scope thereof are explained in detail as follows.

The term "lower" is intended to mean 1 to 6, preferably 1 to 4 carbon atom(s), unless otherwise indicated.

40 Suitable "one or two amino acid(s) residue" means a bivalent residue derived from one or two amino acid(s), and such amino acid may be neutral amino acid such as glycine (Gly), D- or L- alanine (Ala),  $\beta$ -alanine ( $\beta$ -Ala), D- or L- valine (Val), D- or L- leucine (Leu), D- or L- isoleucine (Ile), D- or L- serine (Ser), D- or L- threonine (Thr), D- or L- cysteine (Cys), D- or L- methionine (Met), D- or L- phenylalanine (Phe), D- or L- tryptophan (Trp), D- or L- tyrosine (Tyr), D- or L- proline (Pro), D- or L- 4-hydroxyproline (Hyp), D- or L- pyroglutamic acid (pGlu), acidic amino acid such as D- or L- glutamic acid (Glu) D- or L- aspartic acid (Asp), D- or L-  $\beta$ -aspartic acid ( $\beta$ Asp), D- or L- glutamine (Gln), D- or L- asparagine (Asn), and basic amino acid such as D- or L- lysine (Lys), D- or L- arginine (Arg), D- or L- histidine (His), D- or L- ornithine (Orn), and combination of two of such amino acid, whose side chains, which are amino, hydroxy, thiol or carboxy groups, may be substituted by the suitable substituent(s) such as di(lower)alkylamino (e.g., dimethylamino, etc.), trihalo(lower)alkoxycarbonyl (e.g., 2,2,2-trichloroethoxycarbonyl, etc.), ar(lower)alkoxycarbonyl (e.g., benzoyloxycarbonyl, etc.), arenesulfonyl (e.g., benzenesulfonyl, toluenesulfonyl, etc.), haloar(lower)alkoxycarbonyl (e.g., o-chlorobenzoyloxycarbonyl, etc.), ar(lower)alkyl (e.g., benzyl, phenethyl, etc.), trihalo(lower)alkyl (e.g., 2,2,2-trichloroethyl, etc.), carboxy(lower)alkanoyl (e.g., carboxypropionyl, etc.), glyceryl,  $\beta$ -alanyl, N-lower alkoxycarbonylglyceryl (e.g., N-t-butoxycarbonylglyceryl, etc.) and N-lower alkoxycarbonyl-  $\beta$ -alanyl (e.g., N-t-butoxycarbonylglyceryl, etc.), or usual protecting group used in the field of amino acid and peptide chemistry such as those mentioned below.

Suitable "an amino acid residue" means a bivalent residue derived from the amino acid as mentioned above.

As to the formula "-Trp(R<sup>2</sup>)-", it means the group R<sup>2</sup> being substituted at 1-position of indole group in tryptophan residue.

Suitable "amino protective group" may include a conventional protective group, which is used in the field of amino acid and peptide chemistry, that is may be ar(lower)alkyl (e.g. trityl, benzhydryl, benzyl, etc.), dinitrophenyl, lower alkoxycarbonyl(lower)alkenyl (e.g. 1-methoxycarbonyl-1-propen-2-yl, etc.), aroyl(lower)-alkenyl (e.g. 1-benzoyl-1-propen-2-yl, etc.), hydroxyar(lower)alkylidene (e.g. 2-hydroxybenzylidene, etc.), silyl compound such as tri(lower)alkylsilyl (e.g. trimethylsilyl, etc.), acyl as mentioned below, or the like.

Suitable "acyl" may include an aliphatic acyl, an aromatic acyl, a heterocyclic acyl and an aliphatic acyl substituted with aromatic or heterocyclic group(s).

The aliphatic acyl may include saturated or unsaturated, acyclic or cyclic ones, such as carbamoyl, lower alkanoyl (e.g. formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, etc.), lower alkanesulfonyl (e.g. mesyl, ethanesulfonyl, propanesulfonyl, etc.), lower alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, t-butoxycarbonyl, etc.), lower alkenoyl (e.g. acryloyl, methacryloyl, crotonoyl, etc.), (C<sub>3</sub>-C<sub>7</sub>)-cycloalkanecarbonyl (e.g. cyclohexanecarbonyl, etc.), amidino, protected carboxycarbonyl such as lower alkoxalyl (e.g. methoxyalyl, ethoxalyl, t-butoxalyl, etc.), and the like.

The aromatic acyl may include aroyl (e.g. benzoyl, toluoyl, xyloyl, etc.), arenesulfonyl (e.g. benzenesulfonyl, tosyl, etc.), and the like.

The heterocyclic acyl may include heterocyclecarbonyl (e.g. furoyl, thenoyl, nicotinoyl, isonicotinoyl, thiazolylcarbonyl, thiadiazolylcarbonyl, tetrazolylcarbonyl, morpholinocarbonyl, etc.), and the like.

The aliphatic acyl substituted with aromatic group(s) may include ar(lower)alkanoyl such as phenyl-(lower)alkanoyl (e.g. phenylacetyl, phenylpropionyl, phenylhexanoyl, etc.), ar(lower)alkoxycarbonyl such as phenyl(lower)alkoxycarbonyl (e.g. benzyloxycarbonyl, phenethyloxycarbonyl, etc.), phenoxy(lower)alkanoyl (e.g. phenoxyacetyl, phenoxypropionyl, etc.), and the like.

The aliphatic acyl substituted with heterocyclic group(s) may include thienylacetyl, imidazolylacetyl, furylacetyl, tetrazolylacetyl, thiazolylacetyl, thiadiazolylacetyl, thienylpropionyl, thiadiazolylpropionyl, and the like.

These acyl groups may be further substituted with one or more suitable substituents such as carboxy, lower alkyl (e.g. methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl, hexyl, etc.), halogen (e.g. chlorine, bromine, iodine, fluorine), carbamoyl, amino which may be substituted by suitable substituent(s) such as lower alkanoyl (e.g. formyl, acetyl, propionyl, etc.), ar(lower) alkyl (e.g. benzyl, etc.), lower alkyl (e.g. methyl, ethyl, propyl, isopropyl, butyl, t-butyl, etc.), lower alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, etc.), carboxy(lower)alkyl (e.g. carboxymethyl, carboxyethyl, etc.), protected carboxy(lower)-alkyl (e.g. t-butoxycarbonylmethyl, etc.) and the like.

Suitable "carbamoyl(lower)alkyl" may include carbamoylmethyl, carbamoylethyl, carbamoylpropyl, and the like.

Suitable "carboxy(lower)alkyl" may include carboxymethyl, carboxyethyl, carboxypropyl, and the like.

Suitable "protected carboxy(lower)alkyl" means the above-mentioned carboxy(lower)alkyl, in which the carboxy group is protected by a conventional protective group such as esterified carboxy group. Preferred example of the ester moiety thereof may include lower alkyl ester (e.g. methyl ester, ethyl ester, propyl ester, etc.) and the like.

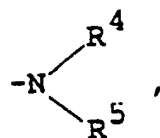
Suitable "aryl" may include phenyl, tolyl, xylyl, naphthyl, and the like.

Suitable "lower alkyl which may have suitable substituent(s)" may include a conventional group, which is used in the field of amino acid and peptide chemistry, such as lower alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, cyclohexyl, etc.), hydroxy(lower)alkyl (e.g. hydroxymethyl, hydroxyethyl, etc.), protected hydroxy(lower)alkyl such as acyloxy(lower) alkyl (e.g. benzyloxycarbonyloxymethyl, benzyloxycarbonyloxyethyl, etc.), substituted or unsubstituted ar(lower)alkyl (e.g., trityl, benzyl, phenethyl, halogen substituted ar(lower)alkyl such as o-fluorobenzyl, p-chlorobenzyl, p-nitrobenzyl, etc.), heterocyclic(lower)-alkyl, for instance, pyridyl(lower)alkyl (e.g., 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, etc.) and the like.

Suitable "lower alkyl" may include a straight or branched one such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, and the like.

Suitable "ar(lower)alkyl" may include trityl, benzhydryl, benzyl, phenethyl, and the like.

Suitable group of the formula :



5

in which R<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-condensed lower alkylene, may include 1-indoliny, 2-isoindoliny, 1,2,3,4-tetrahydroquinolin-1-yl, 1,2,3,4-tetrahydroisoquinolin-2-yl, and the like.

10 Suitable "amino protective group containing a protected carboxy" may include a protected carboxycarbonyl (e.g. methoxalyl, ethoxalyl, t-butoxalyl, etc.), and the like.

Suitable "amino protective group containing a carboxy" may include carboxycarbonyl, and the like.

Suitable "amino protective group containing an amino group which is substituted by an amino protective group and additionally a protected carboxy(lower)alkyl or an ar(lower)alkyl" may include N-lower  
15 alkoxy carbonyl-N-lower alkoxy carbonyl(lower)alkylamino(lower)alkanoyl (e.g. N-t-butoxycarbonyl-N-t-butoxycarbonylmethylaminoacetyl, etc.), N-lower alkoxy carbonyl-N-ar(lower)alkylamino(lower)alkanoyl (e.g. N-t-butoxycarbonyl-N-benzylaminoacetyl, etc.), and the like.

Suitable "an amino protective group containing an amino group which is substituted by a carboxy-(lower)alkyl or an ar(lower)alkyl" may include carboxy(lower)alkylamino(lower)alkanoyl (e.g. carboxymethylaminoacetyl, etc.), ar(lower)alkylamino(lower)alkanoyl (e.g. benzylaminoacetyl, etc.), and the like.  
20

Suitable "hydroxy(lower)alkyl" may include hydroxymethyl, hydroxyethyl, hydroxypropyl, and the like.

Suitable "protected hydroxy(lower)alkyl" means the above-mentioned hydroxy(lower)alkyl, in which the hydroxy group is protected by a conventional protective group. Preferred example of the protective group may include aforesaid acyl (e.g. benzyloxycarbonyl, etc.), ar(lower)alkyl (e.g. benzyl, etc.) and the like.

25 Suitable "one or two amino acid(s) residue containing a hydroxy group, an amino group, an imino group or a carboxy group" may include bivalent residue of an amino acid such as Thr, His, Lys, Orn, Trp, Arg, Glu, and the like, and the bivalent residue of two amino acid(s) in which one of said amino acids is Thr, His, Lys, Orn, Trp, Arg, Glu, and the like.

Suitable "one or two amino acid(s) residue containing a protected hydroxy group, a protected amino  
30 group, a protected imino group or a protected carboxy group" means the above-mentioned group, in which the hydroxy, amino, imino or carboxy group is protected by a conventional group used in the field of the amino acid chemistry such as the ar(lower)alkyl or amino-protected group mentioned above.

Suitable "one or two amino acid(s) residue which is substituted by acyl having amino" means a bivalent residue derived from one or two amino acid(s), whose side chain is substituted by acyl having amino such  
35 as amino(lower)alkanoyl (e.g. aminoacetyl, aminopropionyl, etc.).

Suitable "one or two amino acid(s) residue which is substituted by acyl having protected amino" means a bivalent residue derived from one or two amino acid(s), whose side chain is substituted by acyl having protected amino. Such acyl group means the above mentioned group, and is protected by the amino protected group mentioned above.

40 Particularly, the preferred embodiment of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> and A are as follows.

R<sup>1</sup> is hydrogen:- or

acyl, for example, carbamoyl;

lower alkoxy carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, etc.);

lower alkanoyl (e.g. formyl, acetyl, propionyl, butyryl, etc);

45 ar(lower)alkoxy carbonyl such as mono or di or triphenyl(lower)alkoxy carbonyl (e.g. benzyloxycarbonyl, etc.), etc.;

carbamoyl(lower)alkanoyl (e.g. carbamoylactyl, succinamoyl, etc.);

lower alkoxy alyl (e.g. methoxalyl, t-butoxalyl, etc.);

di(lower)alkylamino(lower)alkanoyl (e.g. dimethylaminoacetyl, diethylaminoacetyl, diethylaminopropionyl,  
50 etc.);

N-ar(lower)alkyl-N-lower alkoxy carbonylamino(lower)alkanoyl such as N-mono or di or triphenyl(lower)alkyl-N-lower alkoxy carbonylamino(lower)alkanoyl (e.g. N-benzyl-N-t-butoxycarbonylaminoacetyl, etc.), etc.;

heterocyclic (lower)alkanoyl optionally substituted with acylamino such as tetrazolyl(lower)alkanoyl (e.g. tetrazolylacetyl, etc.), acylaminothiazolyl(lower)alkanoyl which may have acylamino on the alkanoyl moiety,

55 for instance, lower alkanoylaminothiazolyl(lower)alkanoyl (e.g. formamidothiazolylacetyl, etc.), lower alkanoylaminothiazolyl(lower)alkanoyl having lower alkoxy carbonylamino or lower alkanoylamino on the alkanoyl moiety (e.g. 2-formamidothiazolyl-2-t-butoxycarbonylaminoacetyl, 2-formamidothiazolyl-2-acetamidoacetyl, etc.), etc.;

carboxy(lower)alkanoyl (e.g. oxalo, carboxyacetyl, carboxypropionyl, carboxybutyryl, carboxyvaleryl, etc.);  
hydroxy(lower)alkanoyl (e.g. hydroxyacetyl, etc.);

heterocyclic carbonyl such as morpholinecarbonyl (e.g. 4-morpholinecarbonyl, etc.), etc.;

lower alkylcarbamoyl (e.g. methylcarbamoyl, t-butylcarbamoyl, etc.);

5 carboxy(lower)alkylamino(lower)alkanoyl (e.g. carboxymethylaminoacetyl, etc.);

ar(lower)alkylamino(lower)alkanoyl such as mono or di triphenyl(lower)alkylamino(lower)alkanoyl (e.g. benzylaminoacetyl, etc.), etc.;

N-lower alkoxycarbonyl-N-lower alkoxycarbonyl(lower)alkylamino(lower)alkanoyl (e.g. N-t-butoxycarbonyl-N-t-butoxycarbonylmethylaminoacetyl, etc.); and the like:-

10 R<sup>2</sup> is hydrogen;

acyl such as lower alkanoyl (e.g. formyl, acetyl, etc.), arenesulfonyl (e.g. benzenesulfonyl, toluenesulfonyl, etc.), etc.;

carbamoyl(lower)alkyl (e.g. carbamoylmethyl, etc.);

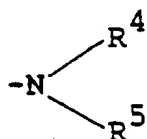
esterified carboxy(lower)alkyl such as lower alkoxycarbonyl(lower)alkyl (e.g. ethoxycarbonylmethyl, etc.),

15 etc.; or

carboxy(lower)alkyl (e.g. carboxymethyl, etc.);

R<sup>3</sup> is ar(lower)alkyl such as mono or di or triphenyl(lower)alkyl (e.g. benzyl, phenethyl, etc.), etc.;

a group of the formula:



25

wherein R<sup>4</sup> is hydrogen;

lower alkyl (e.g. methyl, ethyl, etc.);

hydroxy(lower)alkyl (e.g. hydroxymethyl, hydroxyethyl, etc.); or

30 acyloxy(lower)alkyl such as phenyl(lower)alkoxycarbonyloxy(lower)alkyl (e.g. benzyloxycarbonyloxyethyl, etc.), etc.;

R<sup>5</sup> is aryl (e.g. phenyl, tolyl, xylyl, etc.);

ar(lower)alkyl such as mono or di or triphenyl(lower)alkyl (e.g. benzyl, phenethyl, etc.), etc.; or

haloar(lower)alkyl such as halo-substituted mono or di or triphenyl(lower)alkyl (e.g. fluorobenzyl, etc.),

35 etc.;

R<sup>4</sup> and R<sup>5</sup> are lined together to form benzene-condensed lower alkylene (e.g. 1-indolyl, 1,2,3,4-tetrahydroquinolin-1-yl, 2-isoindolyl, 1,2,3,4-tetrahydroquinolin-2-yl, etc.);

or a group of the formula:

-OR<sup>5</sup>

40 wherein R<sup>5</sup> is lower alkyl (e.g. methyl, ethyl, propyl, isopropyl, etc.);

ar(lower)alkyl such as mono or di or triphenyl(lower)alkyl (e.g. benzyl, phenethyl, etc.), etc.;

haloar (lower) alkyl such as halo-substituted mono or di or triphenyl(lower)alkyl (e.g. chlorobenzyl, etc.);

lower cycloalkyl(lower)alkyl (e.g. cyclohexylmethyl, etc.);

heterocyclic lower alkyl such as pyridyl(lower)alkyl (e.g. pyridylmethyl, etc.), etc.;

45 A is one or two amino acid residue(s) derived from one or amino acid such as glutamine, serine, asparagine, glutamic acid, threonine, lysine, histidine,  $\beta$ -aspartic acid, ornithine, glycine, tyrosine, tryptophan, hydroxyproline, pyroglutamic acid,  $\beta$ -alanine,

N<sup>5</sup>,N<sup>5</sup>-di(lower)alkylglutamine,

N<sup>5</sup>-trihalo(lower)alkoxycarbonyllysine,

50 N<sup>5</sup>-ar(lower)alkoxycarbonyllysine,

N<sup>7</sup>-arenesulfonylhistidine,

N<sup>5</sup>-ar(lower)alkoxycarbonylornithine,

R<sup>5</sup>-haloar(lower)alkoxycarbonyllysine,

O<sup>3</sup>-ar(lower)alkylthreonine, N-lower alkylthreonine,

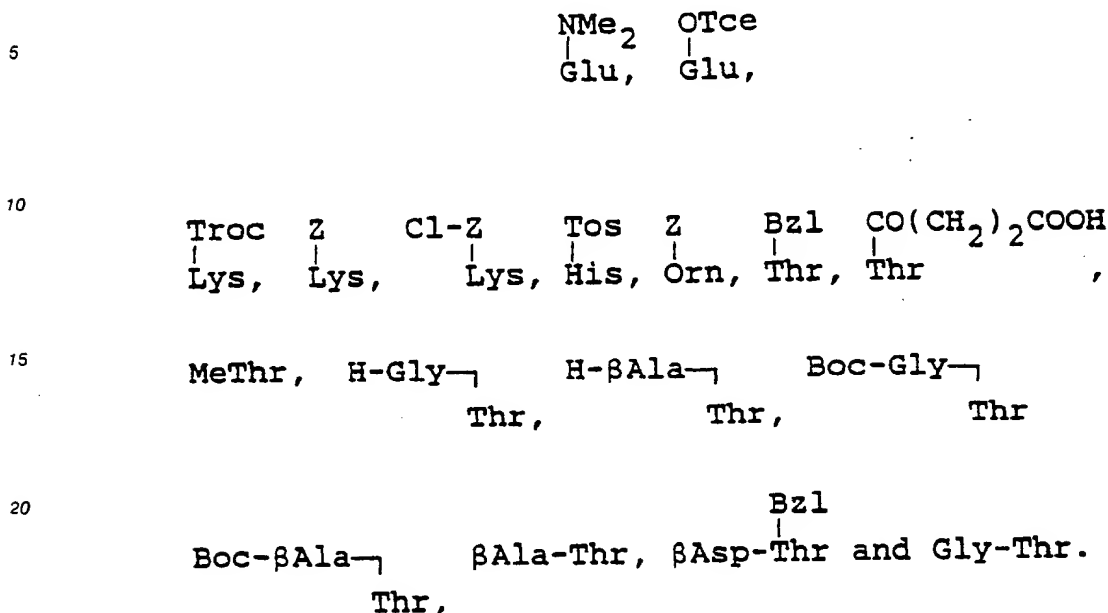
55 O<sup>5</sup>-trihalo(lower)alkyl glutamate,

O<sup>3</sup>-carboxy(lower)alkanoylthreonine,

O<sup>3</sup>-glycylthreonine, O<sup>3</sup>- $\beta$ -alanylthreonine,

O<sup>3</sup>-(N-lower alkoxycarbonylglycyl)threonine

O<sup>3</sup>-(N-lower alkoxy carbonyl-β-alanyl)threonine, etc., more preferably  
Gln, Ser, Asn, Thr, D-Gln, Lys, His, βAsp, Orn, Gly, Tyr, D-Trp, Hyp, pGlu, Glu,



(to be continued to the next page)

The processes for preparing the object compound (I) are explained in detail in the following.

### 35 Process 1

The object compound (Ia) or a salt thereof can be prepared by reacting a compound (II) or its reactive derivative at the carboxy group or a salt thereof with a compound (III) or its reactive derivative at the amino group or a salt thereof.

40 Suitable reactive derivative at the amino group of the compound (III) may include Schiff's base type imino or its tautomeric enamine type isomer formed by the reaction of the compound (III) with a carbonyl compound such as aldehyde, ketone or the like; a silyl derivative formed by the reaction of the compound (III) with a silyl compound such as bis(trimethylsilyl)acetamide, mono(trimethylsilyl)acetamide, bis-(trimethylsilyl)urea or the like; a derivative formed by reaction of the compound (III) with phosphorus  
45 trichloride or phosgene, and the like.

Suitable salts of the compound (III) and its reactive derivative can be referred to the ones as exemplified for the compound (I).

Suitable reactive derivative at the carboxy group of the compound (II) may include an acid halide, an acid anhydride, an activated amide, an activated ester, and the like. Suitable examples of the reactive  
50 derivatives may be an acid chloride; an acid azide; a mixed acid anhydride within acid such as substituted phosphoric acid [e.g. dialkylphosphoric acid, phenylphosphoric acid, diphenylphosphoric acid, dibenzylphosphoric acid, halogenated phosphoric acid, etc.], dialkylphosphorous acid, sulfurous acid, thiosulfuric acid, sulfuric acid, sulfonic acid [e.g. methanesulfonic acid, etc.], aliphatic carboxylic acid [e.g. acetic acid, propionic acid, butyric acid, isobutyric acid, pivalic acid, pentanoic acid, isopentanoic acid, 2-ethylbutyric  
55 acid, trichloroacetic acid, etc.] or aromatic carboxylic acid [e.g. benzoic acid, etc.]; a symmetrical acid anhydride; an activated amide with imidazole, 4-substituted imidazole, dimethylpyrazole, triazole or tetrazole; or an activated ester [e.g. cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl [(CH<sub>3</sub>)<sub>2</sub>N=CH-] ester, vinyl ester, propargyl ester, p-nitrophenyl ester, 2,4-dinitrophenyl ester, trichlorophenyl ester,

pentachlorophenyl ester, mesylphenyl ester, phenylazophenyl ester, phenyl thioester, p-nitrophenyl thioester, p-cresyl thioester, carboxymethyl thioester, pyranil ester, pyridyl ester, piperidyl ester, 8-quinolyl thioester, etc.], or an ester with a N-hydroxy compound [e.g. N,N-dimethylhydroxylamine, 1-hydroxy-2-(1H)-pyridone, N-hydroxysuccinimide, N-hydroxyphthalimide, 1-hydroxy-1H-benzotriazole, etc.], and the like.

5 These reactive derivatives can optionally be selected from them according to the kind of the compound (II) to be used.

Suitable salts of the compound (II) and its reactive derivative may be a base salt such as an alkali metal salt [e.g. sodium salt, potassium salt, etc.], an alkaline earth metal salt [e.g. calcium salt, magnesium salt, etc.], an ammonium salt, an organic base salt [e.g. trimethylamine salt, triethylamine salt, pyridine salt, 10 picoline salt, dicyclohexylamine salt N,N'-dibenzylethylenediamine salt, etc.], or the like, and an acid addition salt as exemplified for the compound [I].

The reaction is usually carried out in a conventional solvent such as water, alcohol [e.g. methanol, ethanol, etc.], acetone, dioxane, acetonitrile, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not 15 adversely influence the reaction. These conventional solvent may also be used in a mixture with water.

In this reaction, when the compound (II) is used in a free acid form or its salt form, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide; N-cyclohexyl-N'-morpholinoethylcarbodiimide; N-cyclohexyl-N'-(4-diethylaminocyclohexyl)carbodiimide; N,N'-diethylcarbodiimide, N,N'-diisopropylcarbodiimide; N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; N,N'-carbonylbis-(2-methylimidazole); pentamethyleneketene-N-cyclohexylimine; diphenylketene-N-cyclohexylimine; ethoxyacetylene; 1-alkoxy-1-chloroethylene; trialkyl phosphite; ethyl polyphosphate; isopropyl polyphosphate; phosphorus oxychloride (phosphoryl chloride); phosphorus trichloride; diphenyl phosphorylazide; thionyl chloride; oxalyl chloride; lower alkyl haloformate [e.g. ethyl chloroformate, isopropyl chloroformate, etc.]; triphenylphosphine; 2-ethyl-7-hydroxybenzisoxazolium salt; 2-ethyl-5-(m-sulfonphenyl)isoxazolium hydroxide intramolecular salt; 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole; so-called Vilsmeier reagent prepared by the reaction of N,N-dimethylformamide with thionyl chloride, phosgene, trichloromethyl chloroformate, phosphorus oxychloride, etc.; or the like.

The reaction may also be carried out in the presence of an inorganic or organic base such as an alkali metal bicarbonate, tri(lower)alkylamine, pyridine, N-(lower)alkylmorpholine, N,N-di(lower)alkylbenzylamine, 30 or the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

## 35 Process 2

The object compound (Ib) or a salt thereof can be prepared by subjecting a compound (Ia) or a salt thereof to elimination reaction of the amino-protective group.

Suitable salts of the compounds (Ia) and (Ib) can be referred to the ones as exemplified for the 40 compound (I).

This reaction is carried out in accordance with a conventional method such as hydrolysis, reduction or the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g. sodium, 45 potassium, etc.], an alkaline earth metal [e.g. magnesium, calcium, etc.], the hydroxide or carbonate or bicarbonate thereof, hydrazine, trialkylamine [e.g. trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, or the like.

Suitable acid may include an organic acid [e.g. formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], an inorganic acid [e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, 50 hydrogen chloride, hydrogen bromide, hydrogen fluoride, etc.] and an acid addition salt compound [e.g. pyridine hydrochloride, etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g. trichloroacetic acid, trifluoroacetic acid, etc.] or the like is preferably carried out in the presence or cation trapping agents [e.g. anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, an alcohol [e.g. methanol, ethanol, etc.], methylene chloride, chloroform, tetrachloromethane, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as the solvent. The reaction temperature is not critical and the reaction is usually carried out under cooling to heating.



The reducing method applicable for the elimination reaction may include chemical reduction and catalytic reaction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g. spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g. reduced cobalt, Raney cobalt, etc.], iron catalysts [e.g. reduced iron, Raney iron, etc.], copper catalysts [e.g. reduced copper, Raney copper, Ullman copper, etc.] and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide, or a mixture thereof. Additionally, in case that the above-mentioned acid to be used in chemical reduction are in liquid, they can also be used as a solvent. Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc., or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling to heating.

### Process 3

The object compound (Id) or a salt thereof can be prepared by reacting the compound (Ic) or its reactive derivative at the amino group or a salt thereof with the compound (IV) or its reactive derivative at the carboxy group or a salt thereof.

Suitable salts of the compound (Ic) and its reactive derivative can be referred to the ones as exemplified for the compound (III).

Suitable salts of the compound (IV) and its reactive derivative can be referred to the ones as exemplified for the compound (II).

Suitable salts of the compound (Id) can be referred to the ones as exemplified for the compound (I).

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction conditions [e.g. reactive derivatives, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

### Process 4

The object compound (Ia) or a salt thereof can be prepared by subjecting the compound (Ib) or its reactive derivative at the amino group to introduction reaction of the amino protective group.

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction conditions [e.g. reactive derivatives, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

### Process 5

The object compound (If) or a salt thereof can be prepared by reacting the compound (Ie) or its reactive derivative at the amino group or a salt thereof with the compound (V) or its reactive derivative at the carboxy group or a salt thereof.

Suitable salts of the compound (Ie) and its reactive derivative can be referred to the ones as exemplified for the compound (III).

Suitable salts of the compound (V) and its reactive derivative can be referred to the ones as exemplified for the compound (III).

Suitable salts of the compound (If) can be referred to the ones as exemplified for the compound (I).

This reaction can be carried out in substantially the same manner as Process 1, and therefore the

reaction mode and reaction conditions [e.g. reactive derivatives, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

## 5 Process 6

The object compound (Ih) or a salt thereof can be prepared by subjecting the compound (Ig) or a salt thereof to elimination reaction of the carboxy protective group.

Suitable salt of the compound (Ig) can be referred to the acid addition salt exemplified for the  
10 compound (I) and suitable salt of the compound (Ih) can be referred to the ones as exemplified for the compound (I).

In the present elimination reaction, all conventional methods used in the elimination reaction of the carboxy protective group, for example, hydrolysis, reduction, elimination using Lewis acid, etc. are applicable. When the carboxy protective group is an ester, it can be eliminated by hydrolysis or elimination  
15 using Lewis acid. The hydrolysis is preferably carried out in the presence of a base or an acid.

Suitable base may include, for example, an inorganic base such as alkali metal hydroxide (e.g. sodium hydroxide, potassium hydroxide, etc.), alkaline earth metal hydroxide (e.g. magnesium hydroxide, calcium hydroxide, etc.), alkali metal carbonate (e.g. sodium carbonate, potassium carbonate, etc.), alkaline earth metal carbonate (e.g. magnesium carbonate, calcium carbonate, etc.), alkali metal bicarbonate (e.g. sodium  
20 bicarbonate, potassium bicarbonate, etc.), alkali metal acetate (e.g. sodium acetate, potassium acetate, etc.), alkaline earth metal phosphate (e.g. magnesium phosphate, calcium phosphate, etc.), alkali metal hydrogen phosphate (e.g. disodium hydrogen phosphate, dipotassium hydrogen phosphate, etc.), or the like. and an organic base such as trialkylamine (e.g. trimethylamine, triethylamine, etc.), picoline, N-methylpyrrolidine, N-methylmorpholine, 1,5-diazabicyclo[4.3.0]non-5-one, 1,4-diazabicyclo[2.2.2]octane, 1,5-  
25 diazabicyclo[5.4.0]undecene-5 or the like. The hydrolysis using a base is often carried out in water or a hydrophilic organic solvent or a mixed solvent thereof.

Suitable acid may include an organic acid (e.g. formic acid, acetic acid, propionic acid, etc.) and an inorganic acid (e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, etc.).

The present hydrolysis is usually carried out in an organic solvent, water or a mixed solvent thereof.

The reaction temperature is not critical, and it may suitably be selected in accordance with the kind of  
30 the carboxy protective group and the elimination method.

The elimination using Lewis acid is preferable to eliminate substituted or unsubstituted ar(lower)alkyl ester and carried out by reacting the compound (Ig) or a salt thereof with Lewis acid such as boron trihalide (e.g. boron trichloride, boron trifluoride, etc.), titanium tetrahalide (e.g. titanium tetrachloride, titanium  
35 tetrabromide, etc.), tin tetrahalide (e.g. tin tetrachloride, tin tetrabromide, etc.), aluminum halide (e.g. aluminum chloride, aluminum bromide, etc.), trihaloacetic acid (e.g. trichloroacetic acid, trifluoroacetic acid, etc.) or the like. This elimination reaction is preferably carried out in the presence of cation trapping agents (e.g. anisole, phenol, etc.) and is usually carried out in a solvent such as nitroalkane (e.g. nitromethane, nitroethane, etc.), alkylene halide (e.g. methylene chloride, ethylene chloride, etc.), diethyl ether, carbon  
40 disulfide or any other solvent which does not adversely affect the reaction. These solvents may be used as a mixture thereof.

The reduction elimination can be applied preferably for elimination of the protective group such as halo-(lower)alkyl (e.g. 2-iodoethyl, 2,2,2-trichloroethyl, etc.) ester, ar(lower)alkyl (e.g. benzyl, etc.) ester or the like.

The reduction method applicable for the elimination reaction may include, for example, reduction by  
45 using a combination of a metal (e.g. zinc, zinc amalgam, etc.) or a salt of chromium compound (e.g. chromous chloride, chromous acetate, etc.) and an organic or an inorganic acid (e.g. acetic acid, propionic acid, hydrochloric acid, etc.); and conventional catalytic reduction in the presence of a conventional metallic catalyst (e.g. palladium carbon, Raney nickel, etc.).

The reaction temperature is not critical, and the reaction is usually carried out under cooling, at ambient  
50 temperature or under warming.

## Process 7

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The object compound (Ij) or a salt thereof can be prepared by subjecting the compound (Ii) or a salt thereof to elimination reaction of the amino, hydroxy or carboxy protective group.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the

reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

#### 5 Process 8

The object compound (Ii) or a salt thereof can be prepared by subjecting the compound (Ik) or a salt thereof to elimination reaction of the amino protective group.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the  
10 reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for R<sup>1</sup> and/or lower alkyl which may have suitable substituent(s) for R<sup>4</sup>, R<sup>5</sup>, or R<sup>6</sup> in R<sup>3</sup> is eliminated during the reaction or at the post-treating step of the present process.

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#### Process 9

The object compound (In) or a salt thereof can be prepared by subjecting the compound (Im) or a salt  
20 thereof to elimination reaction of R<sub>a</sub><sup>6</sup>.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for  
25 R<sup>1</sup> and/or R<sup>2</sup> is eliminated during the reaction or at the post-treating step of the present process.

#### Process 10

The object compound (Ip) or a salt thereof can be prepared by subjecting the compound (Io) or a salt  
30 thereof to elimination reaction of the hydroxy protective group.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for  
35 R<sup>1</sup> and/or R<sup>2</sup> is eliminated during the reaction or at the post-treating step of the present process.

#### Process 11

The object compound (Ir) or a salt thereof can be prepared by reacting the compound (Iq) or a salt  
40 thereof with the compound (VI).

This reaction is usually conducted in a conventional solvent which does not adversely influence the reaction such as water, acetic acid, benzene, methanol, ethanol, tetrahydrofuran, dichloromethane, or a  
45 mixture thereof. The reaction temperature is not critical and the reaction is preferably conducted within the range of cooling to warming.

#### Process 12

The object compound (It) or a salt thereof can be prepared by subjecting the compound (Is) or a salt  
50 thereof to elimination reaction of the carboxy protective group.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction  
55 temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for R<sup>1</sup> and/or R<sup>2</sup> and/or lower alkyl which may have suitable substituent(s) for R<sup>4</sup>, R<sup>5</sup> or R<sup>6</sup> in R<sup>3</sup> is eliminated during the reaction or at the post-treating step of the present process.

Process 13

The object compound (li) or a salt thereof can be prepared by subjecting the compound (lj) or a salt thereof to introduction reaction of the amino, hydroxy or carboxy protective group.

The reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction conditions [e.g. solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

Process 14

The object compound (lv) or a salt thereof can be prepared by subjecting the compound (lu) or a salt thereof to elimination reaction of the amino protective group.

This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction conditions [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for R<sup>1</sup> and/or R<sup>2</sup> and/or lower alkyl which may have suitable substituent(s) for R<sup>4</sup>, R<sup>5</sup> or R<sup>6</sup> in R<sup>3</sup> is eliminated during the reaction or at the post-treating step of the present process.

Process 15

The object compound (lx) or a salt thereof can be prepared by subjecting the compound (lw) or a salt thereof to elimination reaction of the amino and/or carboxy protective group.

The reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction condition [e.g. bases, acids, reducing agents, catalysts, solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 2.

The present elimination reaction includes, within its scope, the case that the amino protective group for R<sup>2</sup> and/or lower alkyl which may have suitable substituent(s) for R<sup>4</sup>, R<sup>5</sup> or R<sup>6</sup> in R<sup>3</sup> is eliminated during the reaction or at the post-treating step of the present process.

Process 16

The object compound (lz) or a salt thereof can be prepared by subjecting the compound (ly) or a salt thereof to ring closure reaction.

The reaction may be carried out in the presence of an inorganic or organic acid such as acetic acid, and the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

Process 17

The object compound (lzz) or a salt thereof can be prepared by reacting the compound (lh) or a salt thereof with ammonia.

This reaction can be carried out in substantially the same manner as Process 11, and therefore the reaction conditions [e.g. solvents, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 11.

The compounds obtained by the above processes can be isolated and purified by a conventional method such as pulverization, recrystallization, column chromatography, reprecipitation, or the like.

It is to be noted that the compound (l) and the other compounds may include one or more stereoisomers due to asymmetric carbon atoms, and all of such isomers and mixture thereof are included within the scope of this invention.

The object compounds (l) and pharmaceutically acceptable salts thereof have pharmacological activities such as tachykinin antagonism and the like, and useful for therapeutical treatment and prevention of asthma and the like.

For therapeutic purpose, the compounds (I) and pharmaceutically acceptable salts thereof of the present invention can be used in a form of pharmaceutical preparation containing one of said compounds, as an active ingredient, in admixture with a pharmaceutically acceptable carrier such as an organic or inorganic solid or liquid excipient suitable for oral, parenteral or external administration. The pharmaceutical preparations may be capsules, tablets, dragees, granules, solution, suspension, emulsion, or the like. If desired, there may be included in these preparations, auxiliary substances, stabilizing agents, wetting or emulsifying agents, buffers and other commonly used additives.

While the dosage of the compounds (I) will vary depending upon the age and condition of the patient, an average single dose of about 0.1 mg, 1 mg, 10 mg, 50 mg, 100 mg, 250 mg, 500 mg and 1000 mg of the compound (I) may be effective for treating asthma and the like. In general, amounts between 0.1 mg/body and about 1,000 mg/body may be administered per day.

In order to illustrate the usefulness of the object compound (I), the pharmacological test data of some representative compounds of the compound (I) are shown in the following.

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#### Test methods :

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#### 1. $^3\text{H}$ -Substance P receptor binding

##### (a) Crude lung membrane preparation

Male Hartley strain guinea pigs were sacrificed by decapitation. The trachea and lung were removed and homogenized in buffer (0.25M sucrose, 50mM Tris-HCl pH 7.5, 0.1mM EDTA) by using Polytron (Kinematica). The homogenate was centrifuged (1000xg, 10min) to remove tissue clumps and the supernatant was centrifuged (14000xg 20min) to yield pellets. The pellets were resuspended in buffer (5mM Tris-HCl pH 7.5), homogenized with a teflon homogenizer and centrifuged (14000xg, 20 min) to yield pellets which were referred to as crude membrane fractions. The obtained pellets were stored at  $-70^\circ\text{C}$  until use.

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##### (b) $^3\text{H}$ -Substance P binding to preparative membrane

Frozen crude membrane fractions were thawed and resuspended in Medium 1 (50mM Tris-HCl pH 7.5, 5mM  $\text{MnCl}_2$ , 0.02% BSA, 2 $\mu\text{g/ml}$  chymostatin, 4 $\mu\text{g/ml}$  leupeptin, 40 $\mu\text{g/ml}$  bacitracin.)  $^3\text{H}$ -substance P (1nM) was incubated with 100 $\mu\text{l}$  of the membrane preparation in Medium 1 at  $4^\circ\text{C}$  for 30 minutes in a final volume of 500 $\mu\text{l}$ . At the end of the incubation period, reaction mixture was quickly filtered over a Whatman GF/B glass filter (pretreated with 0.1% polyethylene imine for 3 hours prior to use) under aspiration. The filters were then washed four times with 5 ml of the buffer (50mM Tris-HCl, pH 7.5). The radioactivity was counted in 5 ml of Aquazol-2 in Packard scintillation counter (Packard TRI-CARB 4530).

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#### Test Compounds :

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(a) Boc-Gln-D-Trp(CHO)-Phe-OBzl

(b) Ac-Gln-D-Trp(CHO)-Phe-OBzl

(c) Z-Gln-D-Trp(CHO)-Phe-OBzl

(d) Boc-Asn-D-Trp(CHO)-Phe-OBzl

(e) Boc-Ser-D-Trp(CHO)-Phe-OBzl

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(f) Boc-Glu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-OBzl

(g) Boc-Thr-D-Trp(CHO)-Phe-OBzl

(h) Boc-Gln-D-Trp(CHO)-Phe-NMeBzl

(i) Boc-Thr-D-Trp(CHO)-Phe-NMeBzl

(j) Boc-Glu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-NMeBzl

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(k) Ac-Thr-D-Trp(CHO)-Phe-NMeBzl

(l) Ac-Clu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-NMeBzl

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Test results :	
Test Compounds (1 $\mu$ g/ml)	Inhibition (%)
(a)	100
(b)	100
(c)	93
(d)	99
(e)	99
(f)	100
(g)	100
(h)	100
(i)	100
(j)	100
(k)	100
(l)	100

In the present specification, there are employed the following abbreviations in addition to the abbreviations adopted by the IUPAC-IUB.

Ac : acetyl

AcOH : acetic acid

Ac<sub>2</sub>O : acetic anhydride

Boc : t-butoxycarbonyl

Bzl : benzyl

Bu<sup>t</sup> : t-butyl

Bzl(Cl) : p-chlorobenzyl

Bzl(o-F) : o-fluorobenzyl

cHex : cyclohexyl

Cl-Z : o-chlorobenzyloxycarbonyl

DCC : dicyclohexylcarbodiimide

DMF : N,N-dimethylformamide

Et : ethyl

4N-HCl/DOX : 4N-hydrogen chloride in 1,4-dioxane

HOBT : N-hydroxybenzotriazole

Hyp : 4-hydroxyproline

Me : methyl

NMM : N-methyl morpholine

Ph : phenyl

Pr<sup>i</sup> : isopropyl

Py(2) : 2-pyridyl

Py(3) : 3-pyridyl

Py(4) : 4-pyridyl

Su : succinimido

Tce : 2,2,2-trichloroethyl

TceOH : 2,2,2-trichloroethanol

TFA : trifluoroacetic acid

THF : tetrahydrofuran

Tos : Tosyl (p-toluenesulfonyl)

Tos-Cl : tosyl chloride (p-toluenesulfonyl chloride)

Troc : 2,2,2-trichloroethoxycarbonyl

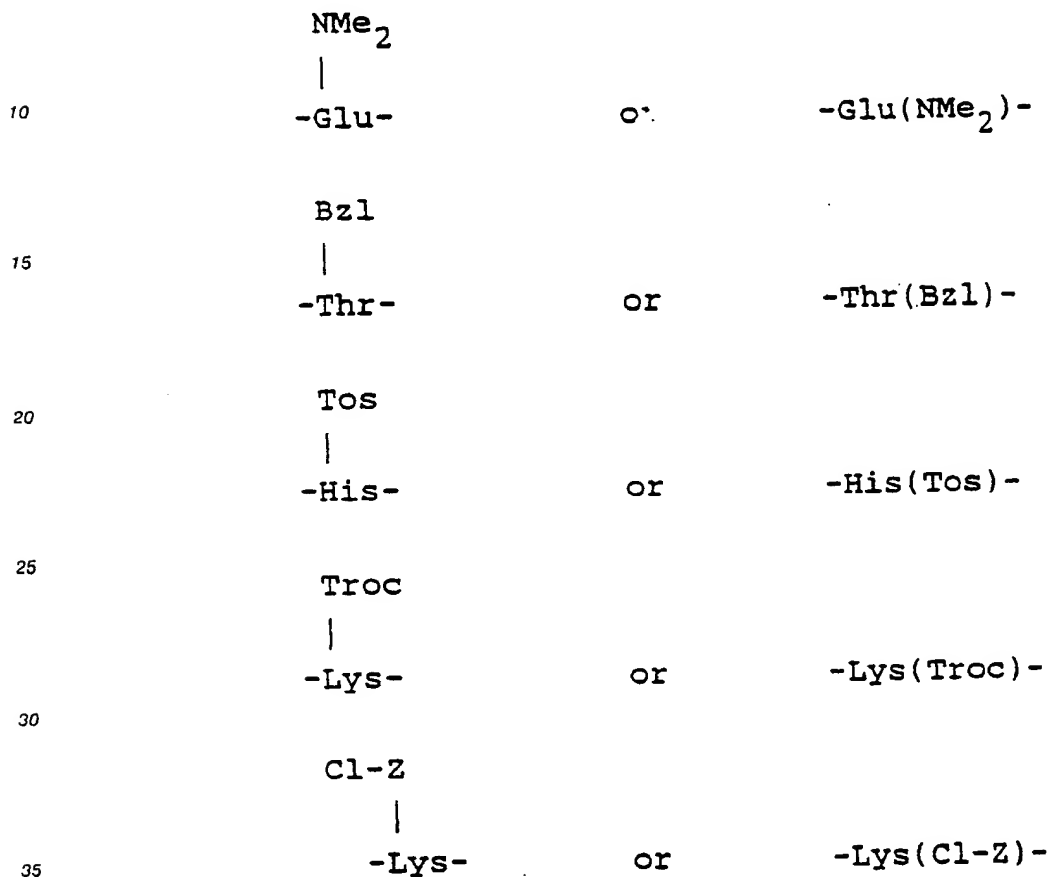
TsOH : p-toluenesulfonic acid (tosic acid)

WSC : 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide

WSC·HCl : 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide·hydrochloride

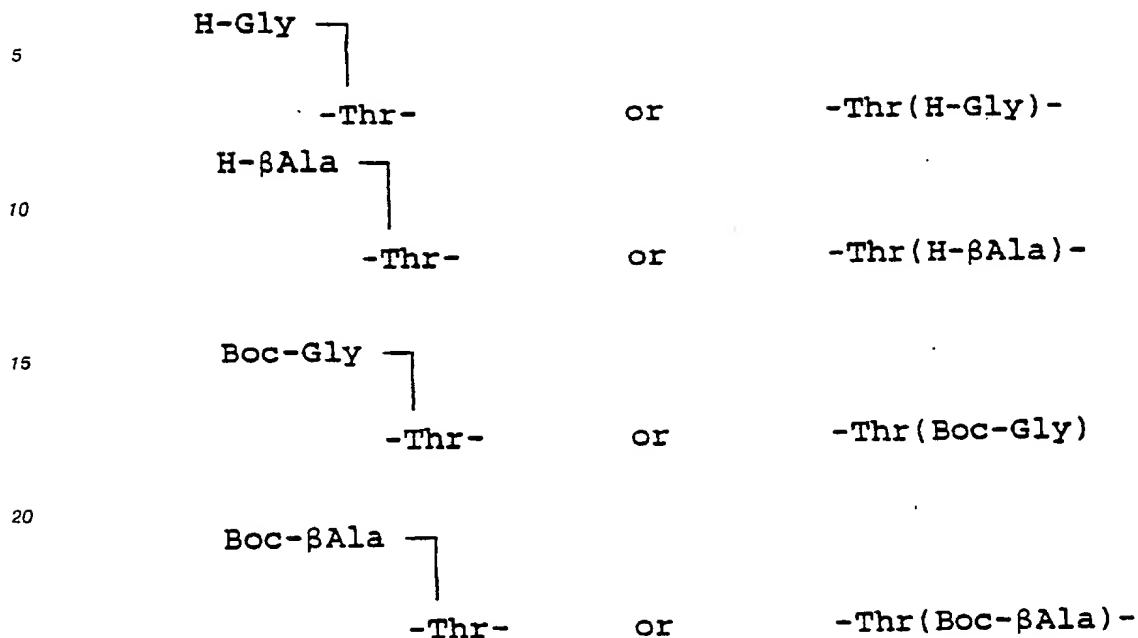
Z : benzyloxycarbonyl

Further, in these examples, substituent groups on side chains in an amino acid residue can be represented by the following formulae.

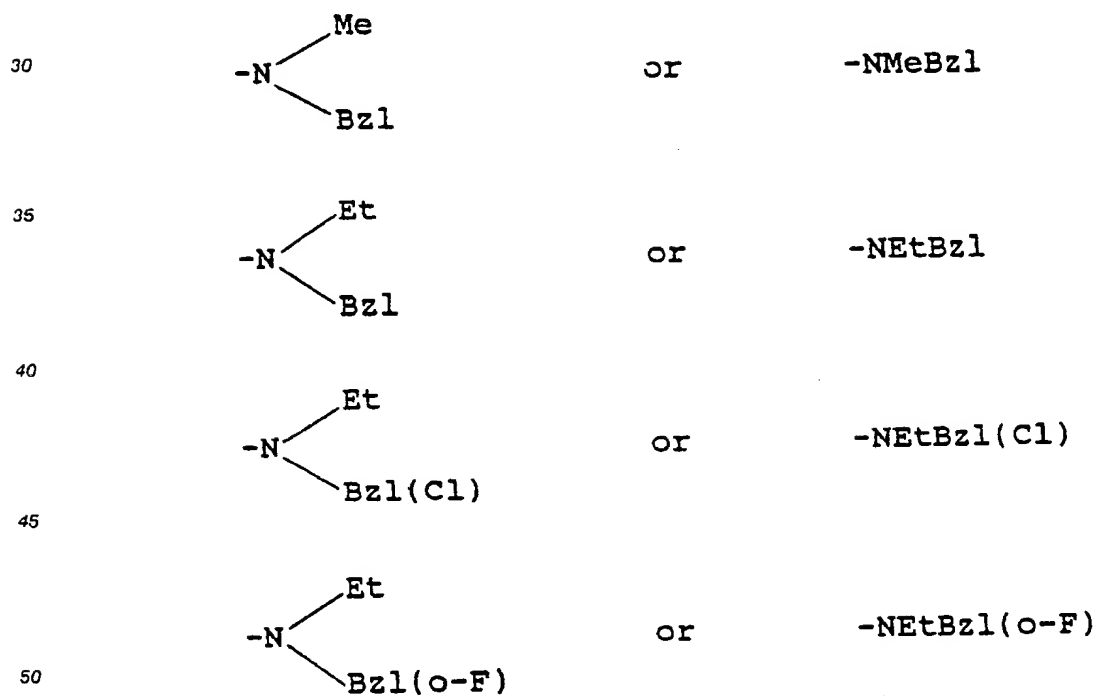


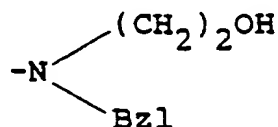
5	$\begin{array}{c} \text{Z} \\   \\ \text{-Lys-} \end{array}$	or	$\text{-Lys(Z)-}$
10	$\begin{array}{c} \text{Z} \\   \\ \text{-Orn-} \end{array}$	or	$\text{-Orn(Z)-}$
15	$\begin{array}{c} \text{CHO} \\   \\ \text{-Trp-} \end{array}$	or	$\text{-Trp(CHO)-}$
20	$\begin{array}{c} \text{Tos} \\   \\ \text{-Trp-} \end{array}$	or	$\text{-Trp(Tos)-}$
25			
30	$\begin{array}{c} \text{CH}_2\text{CO}_2\text{Et} \\   \\ \text{-Trp-} \end{array}$	or	$\text{-Trp(CH}_2\text{CO}_2\text{Et)-}$
35	$\begin{array}{c} \text{CH}_2\text{CO}_2\text{H} \\   \\ \text{-Trp-} \end{array}$	or	$\text{-Trp(CH}_2\text{CO}_2\text{H)-}$
40	$\begin{array}{c} \text{CH}_2\text{CONH}_2 \\   \\ \text{-Trp-} \end{array}$	or	$\text{-Trp(CH}_2\text{CONH}_2\text{)-}$
45			
50	$\begin{array}{c} \text{OTce} \\   \\ \text{-Glu-} \end{array}$	or	$\text{-Glu(OTce)-}$
55	$\begin{array}{c} \text{CO(CH}_2\text{)}_2\text{CO}_2\text{H} \\   \\ \text{-Thr-} \end{array}$	or	$\text{-Thr(CO(CH}_2\text{)}_2\text{CO}_2\text{H)-}$



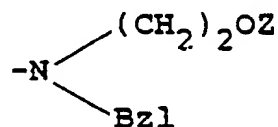
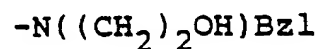


More further, in these examples, the following groups can be represented by the following formulae.

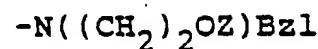




or



or



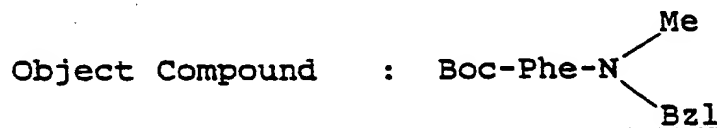
Still more further, in these examples, it is understood that

- Asp -NH<sub>2</sub> means -β-Asp(α-NH<sub>2</sub>)-, and MeThr means N-methylthreonine.  
The following examples are given for purpose of illustrating the present invention in detail.

### Preparation 1

(1)

Starting Compound : Boc-Phe-OH



A solution of Boc-Phe-OH (5.48 g) and NMM (2.09 g) in methylene chloride (50 ml) was cooled to -20°C. To this solution was added dropwise isobutyl chloroformate (2.82 g) maintaining the temperature between -22°C to -20°C in 7 minutes. After stirring the mixture for 20 minutes at the same temperature, the solution was cooled to -35°C and HNMeBzl (2.50 g) was added dropwise to the solution. The reaction mixture was stirred for 2 hours during which period the temperature was gradually raised to -2°C. The solution was washed successively with water (twice), diluted sodium hydrogencarbonate solution (twice), water 0.5N hydrochloric acid (twice), and sodium chloride solution, and dried over magnesium sulfate. After evaporation, the solidified residue was pulverized in hot diisopropyl ether (10 ml), and after cooling, n-hexane (30 ml) was added to the mixture. The crystalline solid was filtered, washed with n-hexane (5 ml x 2), and dried to give Boc-Phe-NMeBzl (6.49 g).

mp : 90-91.5°C

IR (Nujol) : 3380, 1690, 1645 (sh), 1635, 1525 cm<sup>-1</sup>

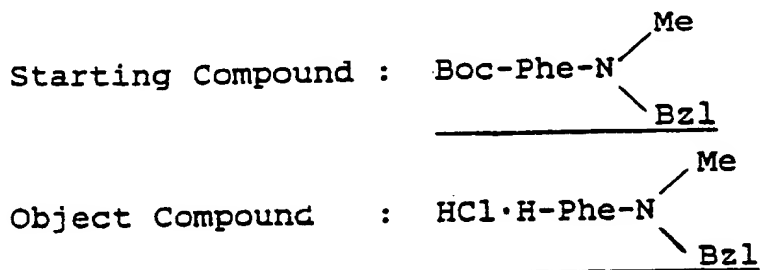
NMR (CDCl<sub>3</sub>, δ) : 1.37 (s) and 1.43 (s) (9H), 2.67 (s) and 2.87 (s) (3H), 3.04 (2H, d, J=7Hz), 4.28 (ABq, J=14Hz) and 4.52 (s) (2H), 4.90 (1H, m), 5.4 (1H, m), 7.0-7.4 (10H)

Elemental analysis.

	Calculated for C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub> :		
Found :	C 71.71, C 72.04,	H 7.66, H 7.65,	N 7.60 N 7.65

$[\alpha]_D^{25} + 19.99^\circ$  (c 1.035, CHCl<sub>3</sub>)

(2)



To an ice-cooled solution of Boc-Phe-NMeBzl (3.0 g) and anisole (3 ml) in methylene chloride (10 ml) was added TFA (12 ml). The solution was stirred for 15 minutes at this temperature and for additional half an hour at room temperature. After evaporation, addition and re-evaporation of 4N-HCl/DOX were repeated twice (4.1 ml and 2.0 ml, respectively). The residue was dissolved in ether (15 ml), and crystallized by seeding. After standing overnight, the crystals were filtered, washed with ether, and dried to give HCl·H-Phe-NMeBzl (2.12 g).

mp : 133-135° C

IR (Nujol) : 3400, 1650 cm<sup>-1</sup>

NMR (CDCl<sub>3</sub>, δ) : 2.43 (s) and 2.70 (s) (3H), 3.5 (2H, m), 4.13 and 4.75 (2H, ABq, J = 14Hz), 5.0 (1H, m), 7.0-7.4 (10H, m), 8.85 (3H, br s)

Elemental Analysis.

	Calculated for C <sub>17</sub> H <sub>20</sub> N <sub>2</sub> O·HCl·1/2H <sub>2</sub> O :		
	C 65.06,	H 7.07,	N 8.93
Found :	C 65.53,	H 6.86,	N 8.90

[α]<sub>D</sub><sup>25</sup> + 57.78° (c 1.066, CHCl<sub>3</sub>)

## Preparation 2

(1)

Starting Compound : Boc-D-Trp-OH

Object Compound : Boc-D-Trp-OBzl

To an ice-cooled solution of Boc-D-Trp-OH (8.61 g) in DMF (100 ml) were added benzyl bromide (7.19 g) and diisopropylethylamine (4.02 g). The solution was stirred for two hours at the same temperature and overnight at room temperature. After evaporation, the residue was extracted with ethyl acetate. The organic layer was washed successively with water, sodium hydrogencarbonate solution, 0.5 hydrochloric acid, and sodium chloride solution, and dried over magnesium sulfate. Evaporation gave Boc-D-Trp-OBzl (10.6 g) as a crystalline mass.

mp : 140° C

IR (Nujol) : 1730, 1690 cm<sup>-1</sup>

NMR (CDCl<sub>3</sub>, δ) : 1.45 (9H, s), 3.32 (2H, d, J = 7Hz), 4.6-5.2 (2H, m), 5.12 (2H, s), 6.85 (1H, d, J = 2Hz), 7.1-7.7 (4H, m), 7.30 (5H, s), 8.13 (1H, br s)

(2)

Starting Compound : Boc-D-Trp-OBzl

Object Compound : Boc-D- $\begin{array}{c} \text{Tos} \\ \text{Trp} \end{array}$ -OBzl

Boc-D-Trp-OBzl (2.0 g) and ethyltrimethylammonium chloride (16.2 mg) were dissolved in methylene chloride (30 ml), and powdered sodium hydroxide (507 mg) was added. To this mixture was added a solution of Tos-Cl (1.45 g) in methylene chloride (5 ml) at room temperature. The reaction mixture was stirred for three and half an hour. After addition of 1N-hydrochloric acid (7.5 ml), the organic layer was separated, washed with sodium chloride solution, dried over magnesium sulfate, and evaporated to give Boc-D-Trp(Tos)-OBzl as an oil (3.23 g).

NMR (CDCl<sub>3</sub>,  $\delta$ ) : 1.43 (9H, s), 2.30 (3H, s), 3.20 (2H, d, J = 6Hz), 4.5-5.2 (2H, m), 5.07 (2H, s), 7.1-8.1 (14H, m)

(3)

Starting compound :  $\text{Boc-D-} \begin{array}{c} \text{Tos} \\ | \\ \text{Trp} \end{array} \text{-OBzl}$

Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{Tos} \\ | \\ \text{Trp} \end{array} \text{-OH}$

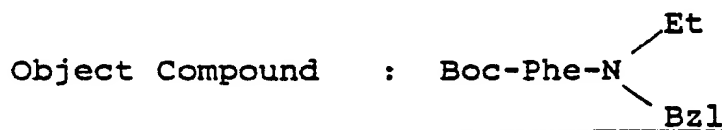
To an ice cooled solution of Boc-D-Trp(Tos)-OBzl (3.23 g) in ethanol (40 ml) was added 1N sodium hydroxide solution (6 ml) at room temperature. The solution was stirred for two hours, during this period two 2 ml portions of 1N sodium hydroxide solution were added. After evaporation of ethanol, and addition of water (50 ml), the solution was extracted once with ether. The aqueous layer was acidified with 1N hydrochloric acid and the resulting oily material was extracted with ethyl acetate, and the extract was washed with sodium chloride, and dried over magnesium sulfate. Evaporation gave Boc-D-Trp(Tos)-OH (2.5 g) as an amorphous solid.

NMR (CDCl<sub>3</sub>,  $\delta$ ) : 1.37 (9H, s), 2.32 (3H, s), 3.3 (2H, m), 4.5-4.8 (1H, m), 4.9-5.3 (1H, m), 7.2-8.3 (8H, m), 8.53 (2H, br s)

### Preparation 3

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Preparation 1-(1).

Starting Compound : Boc-Phe-OH



NMR (CDCl<sub>3</sub>,  $\delta$ ) : 0.93 (3H, t, J = 7Hz), 1.35 (9H, s), 2.8-3.2 (4H, m), 4.1-5.0 (3H, m), 5.1-5.4 (1H, m), 6.8-7.4 (10H, m)

### Preparation 4

Starting Compound : Boc-Phe-OH

Object Compound : Boc-Phe-OCH<sub>2</sub>Py(2)

A mixture of Boc-Phe-OH (1.59 g), 2-pyridinemethanol (0.65 g), DCC (1.24 g) in methylene chloride (30 ml) was stirred for one day at room temperature. The insoluble materials were filtered off, and the filtrate was evaporated. The residue was extracted with ethyl acetate and the organic layer was washed successively with 2% sodium hydrogencarbonate, water and saturated sodium chloride solution, and dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (50 g) and eluted with chloroform. The fractions containing the object compound were combined and evaporated to give Boc-Phe-OCH<sub>2</sub>Py(2) (1.23 g).

IR (Neat) : 3380, 2990, 1740-1710 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 1.32 (9H, s), 2.7-3.2 (2H, m), 4.2-4.5 (1H, m), 5.19 (2H, s), 7.2-7.5 (8H, m), 7.7-8.0 (1H, m), 8.5-8.7 (1H, m)

Object Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OCH<sub>2</sub>CH<sub>6</sub>  
 mp : 78-80 °C

IR (Nujol) : 3350, 1710, 1690, 1650, 1525 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 0.7-1.8 (10H, m), 1.28 (9H, s), 2.6-3.2 (5H, m), 3.87 (2H, d, J=6Hz), 4.0-4.8 (2H, m),  
 5 6.6-6.9 (1H, m), 7.1-7.8 (4H, m), 7.26 (5H, s), 7.9-8.3 (1H, m), 8.53 (1H, br d, J=9Hz), 9.4 (1H, broad)  
 Elemental Analysis.

10

	Calculated for C <sub>33</sub> H <sub>41</sub> N <sub>3</sub> O <sub>6</sub> :		
Found :	C 68.85, C 68.94,	H 7.18, H 7.18,	N 7.30 N 7.30

15

(6)

Starting Compound : Z-D-Trp-OH

Object Compound : Z-D-Trp-Phe-OBzl

mp : 108-111 °C

20

IR (Nujol) : 3450, 3300, 1750, 1700, 1655, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 2.6-3.2 (4H, m), 4.1-4.8 (2H, m), 4.94 (2H, s), 5.13 (2H, s), 6.8-7.8 (21H, m), 8.4-8.7  
 (1H, m), 10.73 (1H, br s)

Elemental Analysis.

25

	Calculated for C <sub>35</sub> H <sub>33</sub> N <sub>3</sub> O <sub>5</sub> :		
Found :	C 73.03, C 72.88,	H 5.78, H 5.83,	N 7.30 N 7.29

30

#### Example 47

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 2.

35

(1)

Starting Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OMe

40

Object Compound : HCl·H-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OMe

IR (Nujol) : 1740, 1710, 1690 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 2.7-3.3 (4H, m), 3.65 (3H, s), 4.0-4.3 (1H, m), 4.4-4.8 (1H, m), 7.24 (5H, s), 7.3-7.5 (2H,  
 m), 7.6-7.9 (2H, m), 8.1-8.5 (1H, m), 8.38 (3H, br s), 9.47 (1H, d, J=8Hz), 9.5 (1H, broad)

45

(2)

Starting Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OPr<sup>i</sup>

50

Object Compound : HCl·H-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OPr<sup>i</sup>

IR (Nujol) : 3350, 1700, 1690 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 1.09 (3H, d, J=7Hz), 1.18 (3H, d, J=7Hz), 2.8-3.3 (4H, m), 3.9-4.3 (1H, m), 4.3-4.7  
 (1H, m), 4.88 (1H, sep, J=7Hz), 7.27 (5H, s), 7.3-7.5 (2H, m), 7.5-7.9 (2H, m), 8.2 (1H, broad), 8.4 (3H, br  
 s), 9.37 (1H, d, J=8Hz), 9.4 (1H, broad)

55

(3)

Starting Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-O(CH<sub>2</sub>)<sub>2</sub>Ph

(2)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OPr}^i$

mp : 100-103 °C

IR (Nujol) : 3340, 1725, 1710, 1690, 1650, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.12 (6H, d, J=6Hz), 1.27 (9H, s), 2.6-3.2 (4H, m), 4.1-4.7 (2H, m), 4.91 (1H, sep), 6.87 (1H, br d, J=9Hz), 7.2=7.6 (3H, m), 7.25 (5H, s), 7.6-7.9 (1H, m), 8.0-8.3 (1H, m), 8.53 (1H, br d, J=9Hz),

9.4 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{29}\text{H}_{35}\text{N}_3\text{O}_6$ :		
Found :	C 66.78, C 66.62,	H 6.76, H 6.47,	N 8.06 N 8.14

(3)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-O(CH}_2)_2\text{Ph}$

mp: 141-142 °C

IR (Nujol) : 3400, 1740, 1720, 1680, 1670, 1525, 1510  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.26 (9H, s), 2.6-3.1 (4H, m), 2.88 (2H, t, J=6Hz), 4.2-4.8 (2H, m), 4.28 (2H, t, J=6Hz), 6.83 (1H, br d, J=9Hz), 7.1-7.6 (3H, m), 7.20 (5H, s), 7.28 (5H, s), 7.6-7.9 (1H, m), 7.9-8.3 (1H, m), 8.53 (1H, br d, J=9Hz), 9.4 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{34}\text{H}_{37}\text{N}_3\text{O}_6$ :		
Found :	C 69.97, C 69.78,	H 6.39, H 6.47,	N 7.20 N 7.26

(4)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl(Cl)}$

mp : 157-158 °C

IR (Nujol) : 3350, 1740, 1720, 1680, 1660, 1545, 1515  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.29 (9H, s), 2.6-3.3 (4H, m), 4.1-4.8 (2H, m), 5.14 (2H, s), 6.93 (1H, br d, J=9Hz), 7.2-7.9 (4H, m), 7.25 (5H, s), 7.43 (4H, s), 8.2 (1H, br s), 8.58 (1H, br d, J=8Hz), 9.4 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{33}\text{H}_{34}\text{ClN}_3\text{O}_6$ :		
Found :	C 65.61, C 65.48,	H 5.67, H 5.56,	N 6.96 N 7.04

(5)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

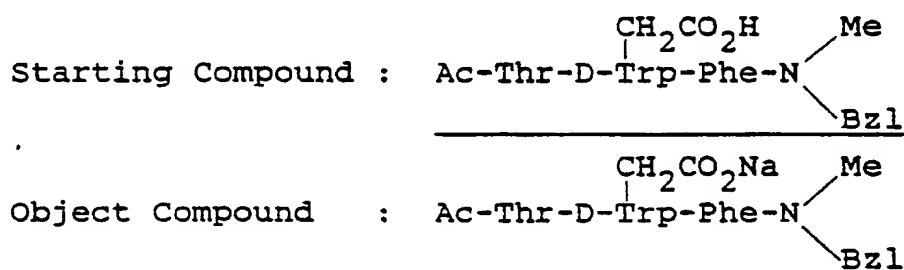
To a solution of Ac-Thr-D-Trp(CH<sub>2</sub>CO<sub>2</sub>Et)-Phe-NMeBzl (0.89 g) in ethanol (25 ml) was added 0.1 N sodium hydroxide solution (14.3 ml) under ice-cooling. After stirring two hours, 0.1 N sodium hydroxide solution (2.0 ml) was added and the mixture was stirred for additional two hours. The ethanol was evaporated and the solution was extracted twice with ethyl acetate. The aqueous layer was acidified with 1N

hydrochloric acid and extracted twice with ethyl acetate. The extract was washed with sodium chloride solution and concentrated to give Ac-Thr-D-Trp(CH<sub>2</sub>CO<sub>2</sub>H)-Phe-NMeBzl as an amorphous solid (0.90 g).

IR (Nujol) : 3300, 1730, 1660 (sh), 1645, 1630 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.84 (3H, d, J = 6Hz), 1.86 (3H, s), 2.7-3.0 ((7H, m), 3.3 (1H, m), 3.8 (1H, m), 4.05-4.2 (2H, m), 4.35-5.0 (3H, m), 4.82 (2H, s), 6.9-7.3 (9H, m), 7.20 (5H, s), 7.45-7.9 (3H, m), 8.4-8.6 (1H, m), 12.7 (1H, br s)

#### Example 45



Ac-Thr-D-Trp(CH<sub>2</sub>CO<sub>2</sub>H)-Phe-NMeBzl (0.509 g) was dissolved in a mixed solvent of acetone (8 ml) and THF (6 ml) and the insoluble material was filtered off. To the solution was added sodium 2-ethyl hexanoate (129 ml) at room temperature. The solution was concentrated to one-third volume and ether (10 ml) was added thereto. After stirring for an hour, the precipitates were collected, washed with ether and dried under vacuum to give Ac-Thr-D-Trp(CH<sub>2</sub>CO<sub>2</sub>Na)-Phe-NMeBzl (0.55 g) as an amorphous solid.

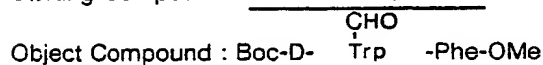
IR (Nujol) : 3300, 1660 (sh), 1640, 1540 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.03 (3H, d, J = 6Hz), 1.93 (3H, s), 2.46 and 2.64 (3H, s), 2.5-2.6 (2H, m), 3.15 (2H, m), 3.8-4.4 (6H, m), 4.60 (2H, s), 6.7-7.4 (15H, m)

#### Example 46

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 1.

(1)



mp : 114-116 °C

IR (Nujol) : 3320, 1740, 1710, 1700, 1680, 1660, 1545, 1525 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.25 (9H, m), 2.6-3.3 (4H, m), 3.65 (3H, s), 4.1-4.8 (2H, m), 6.83 (1H, br d, J=9Hz), 7.2-7.6 (3H, m), 7.24 (5H, s), 7.6-7.9 (1H, m), 8.0-8.4 (1H, m), 8.54 (1H, br d, J=9Hz), 9.4 (1H, broad)

Elemental Analysis.

Calculated for C <sub>27</sub> H <sub>31</sub> N <sub>3</sub> O <sub>6</sub> :			
Found :	C 65.71,	H 6.33,	N 8.51
	C 65.82,	H 6.19,	N 8.45

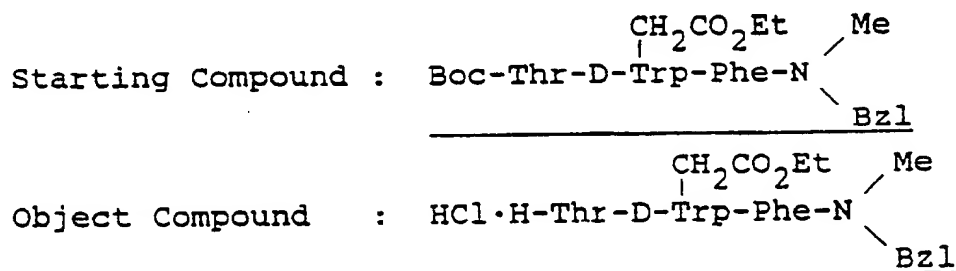
NMR (CDCl<sub>3</sub>, δ) : 1.04 (3H, d, J=6Hz), 1.23 (3H, t, J=7Hz), 1.35 (9H, s), 2.61 and 2.73 (3H, s), 2.85 (2H, d, J=6Hz), 3.23 (2H, d, J=6Hz), 4.08 (2H, q, J=7Hz), 3.8-4.5 (5H, m), 4.71 (2H, s), 4.7 (1H, m), 4.95 (1H, m), 5.41 (1H, d, J=6Hz), 6.7-7.3 (16H, m), 7.4-7.6 (1H, m)

5

Example 42

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 15.

10



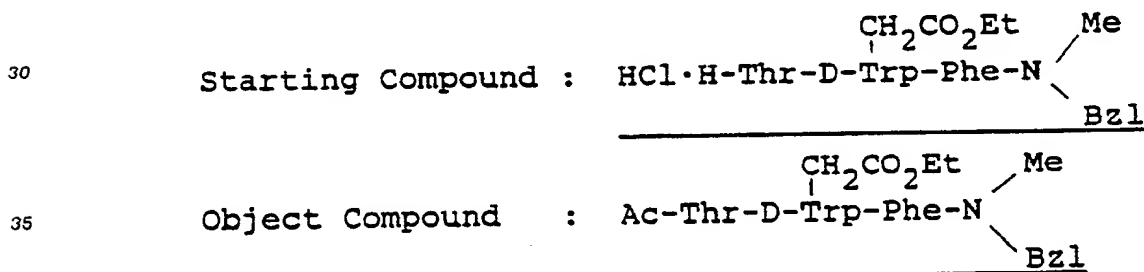
15

20

Example 43

25

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 17.



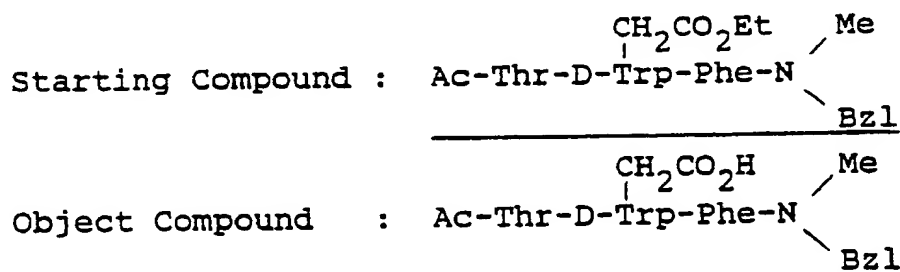
30

35

40 NMR (DMSO-d<sub>6</sub>, δ) : 0.85 (3H, d, J=6Hz), 1.18 (3H, t, J=6Hz), 1.87 (3H, s), 2.74 and 2.81 (3H, s), 2.7-3.1 (4H, m), 3.27 (1H, m), 3.8 (1H, m), 4.1 (1H, m), 4.10 (2H, q, J=6Hz), 4.3-5.1 (4H, m), 4.92 (2H, s), 6.9-7.35 (9H, m), 7.20 (5H, s), 7.5-7.9 (3H, m), 8.5 (1H, m).

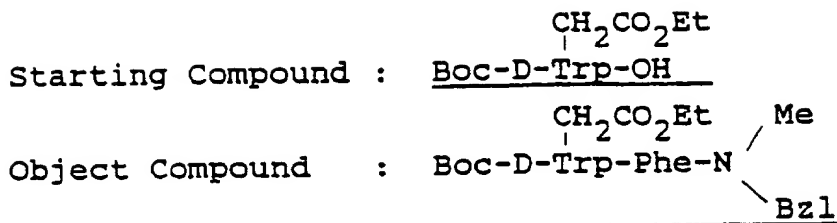
45 Example 44

50



55





mp : 91-104 °C

IR (Nujol) : 3300, 3250, 1760, 1740, 1705, 1670, 1620  $\text{cm}^{-1}$

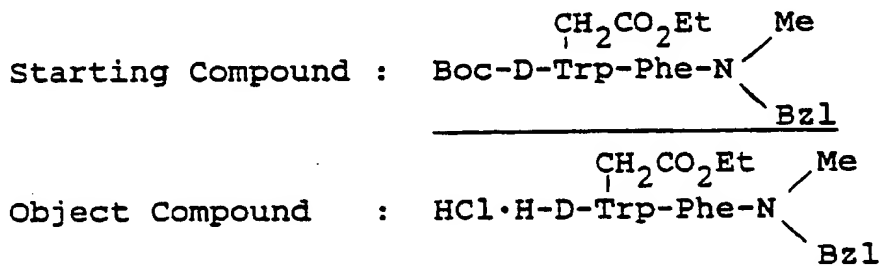
NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.95 and 1.00 (3H, t,  $J=7\text{Hz}$ ), 1.40 (9H, s), 2.54 and 2.73 (3H, s), 2.6-2.8 (2H, m), 3.23 (2H, d,  $J=5\text{Hz}$ ), 4.16 (2H, q,  $J=7\text{Hz}$ ), 4.23 and 4.53 (2H, ABq,  $J=15\text{Hz}$ ), 4.5 (1H, m), 4.70 (2H, s), 4.9-5.2 (2H, m), 6.5-6.7 (1H, m), 6.8-7.3 (14H, m), 7.5-7.7 (1H, m).

Elemental analysis.

	Calculated for $\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_6$ :		
Found :	C 69.35, C 69.14,	H 6.92, H 6.98,	N 8.74 N 8.73

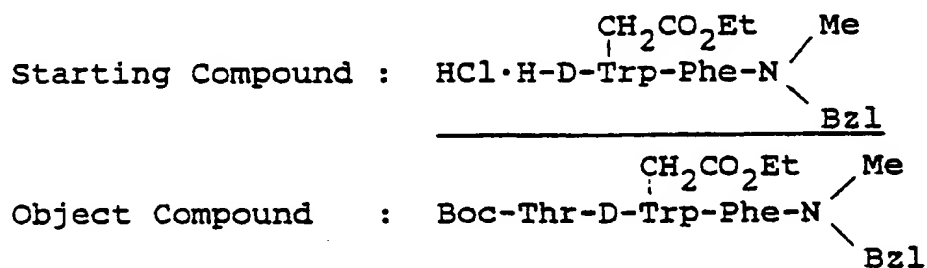
#### Example 40

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 4.



#### Example 41

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 13.



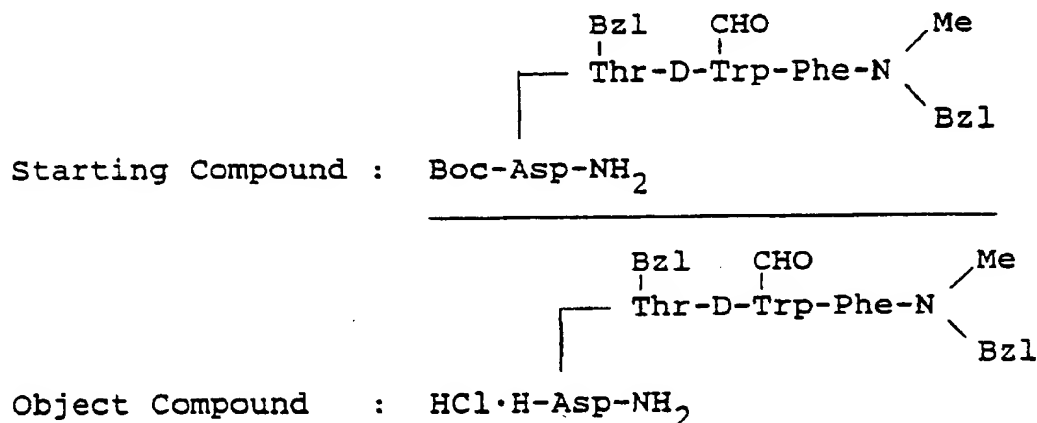
successively with water, 2% hydrochloric acid, water and saturated sodium chloride solution, and dried over magnesium sulfate. After evaporation, the residue was dissolved in DMF (10 ml). To the solution, pyridinium chloride (1.16 g) was added and the mixture was stirred for an hour. After evaporation the residue was solidified with water, filtered, washed with water, and dried. The powder was subjected to column chromatography on silica gel (20 g) and eluted with a mixture of chloroform and methanol (9:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with diethyl ether and filtered. The powder was dissolved in a mixture of chloroform and methanol. To the solution was added 4N-HCl/DOX (0.25 ml) and evaporated. The residue was pulverized with diethyl ether, filtered, washed with diethyl ether and dried to give Ac-His-D-Trp(CHO)-Phe-NMeBzl•HCl (0.31 g).

mp : ~150 °C (dec.)

IR (Nujol) : 3270 (broad), 1710-1630 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.77 (3H, s), 2.5-3.1 (6H, m), 2.78 (s) and 2.85 (s)(3H), 4.1-5.1 (5H, m), 6.9-7.4 (13H, m), 7.4-7.5 (1H, m), 7.5-7.8 (1H, m), 7.8-8.3 (3H, m), 8.5-8.9 (1H, m), 8.89 (1H, s), 9.3 (1H, broad), 14.4 (2H, broad)

### Example 38



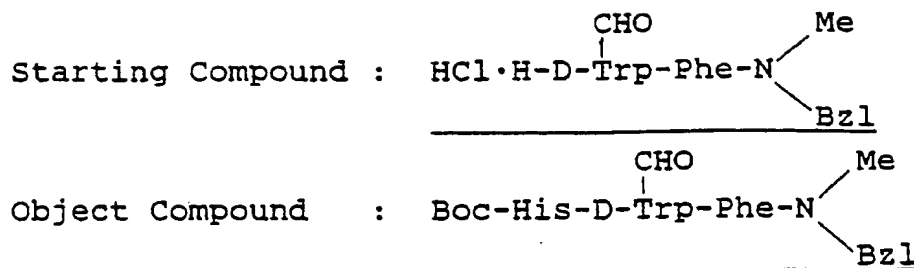
To a mixture of Boc-β-Asp(α-NH<sub>2</sub>)-Thr(Bzl)-D-Trp(CHO)-Phe-NMeBzl (0.92 g) and anisole (1 ml) was added 4N-HCl/DOX (10 ml) at 5 °C. The mixture was stirred for ten minutes under ice-cooling, and for an hour at room temperature. After evaporation, the residue was pulverized with diisopropyl ether, filtered, washed with diisopropyl ether and dried to give HCl•H-β-Asp(α-NH<sub>2</sub>)-Thr(Bzl)-D-Trp(CHO)-Phe-NMeBzl (0.81 g).

IR (Nujol) : 3300 (broad), 1690, 1640 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.85 (3H, d, J = 6Hz), 2.5-3.1 (6H, m), 2.77 (s) and 2.85 (s)(3H), 3.5-5.2 (9H, m), 6.9-7.4 (17H, m), 7.4-7.6 (2H, m), 7.6-7.9 (2H, m), 7.9-8.4 (6H, m), 8.79 (1H, br t, J = 8Hz), 9.2 (1H, broad)

### Example 39

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 3.

Example 36

15 A solution of Boc-His(Tos)-OH (0.82 g) in DMF (10 ml) was cooled at  $-15^{\circ}\text{C}$ . To the solution, NMM (0.22 ml) and isobutyl chloroformate (0.26 ml) were added successively and the mixture was stirred for ten minutes. On the other and, a solution of  $\text{HCl} \cdot \text{H-D-Trp(CHO)-Phe-NMeBzl}$  in DMF (10 ml) was cooled at  $-15^{\circ}\text{C}$  and thereto was added NMM (0.22 ml). This solution was added to the above mentioned mixture and stirred for an hour at  $-15^{\circ}\text{C}$ . After evaporation and extraction with ethyl acetate, the organic layer was

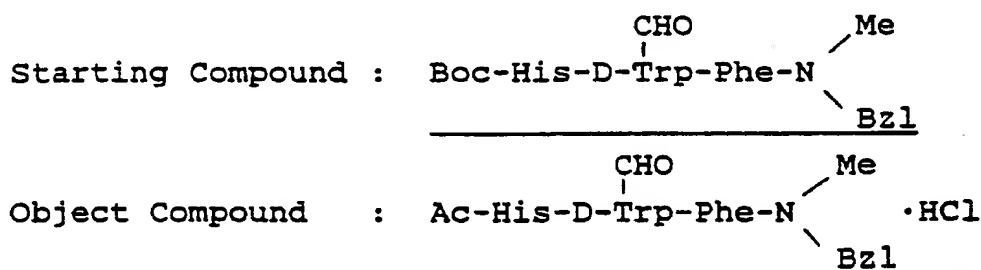
20 washed successively with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride solution, and dried over magnesium sulfate to give Boc-His(Tos)-D-Trp(CHO)-Phe-NMeBzl. After evaporation, the residue was dissolved in DMF (20 ml). To the solution, pyridinium chloride (2.18 g) was added under stirring at room temperature. After an hour, additional pyridinium chloride (0.5 g) was added and stirred for additional fifty minutes. After evaporation and extraction with ethyl acetate, the organic

25 layer was washed successively with water, 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water, saturated sodium chloride solution and dried over magnesium sulfate. After evaporation, the residue was subjected to column chromatography on silica gel (60 g) and eluted with a mixture of chloroform and methanol (20:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with a mixture of ethanol, diethyl ether and n-hexane. The powder was filtered, washed with

30 n-hexane and dried to give Boc-His-D-Trp(CHO)-Phe-NMeBzl (1.04 g).  
 mp :  $-133^{\circ}\text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1640  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 2.5-3.1 (6H, m), 2.76 (s) and 2.84 (s)(3H), 3.9-5.1 (5H, m), 6.5-6.9 (1H, m), 6.56 (1H, s), 6.9-7.7 (14H, m), 7.45 (1H, s), 7.7-8.3 (2H, m), 8.6-8.8 (1H, m), 9.2 (1H, broad), 11.6 (1H, br s)

Example 37

50 To an ice-cooled solution of Boc-His-D-Trp(CHO)-Phe-NMeBzl (0.70 g) and anisole (0.7 ml) in methylene chloride (5 ml) was added 4N-HCl/DOX (5 ml). The solution was stirred for an hour at room temperature. After evaporation, the residue was pulverized with diisopropyl ether, filtered, washed with diisopropyl ether and dried to give  $2\text{HCl} \cdot \text{H-His-D-Trp(CHO)-Phe-NMeBzl}$ . The powder (0.70 g) was

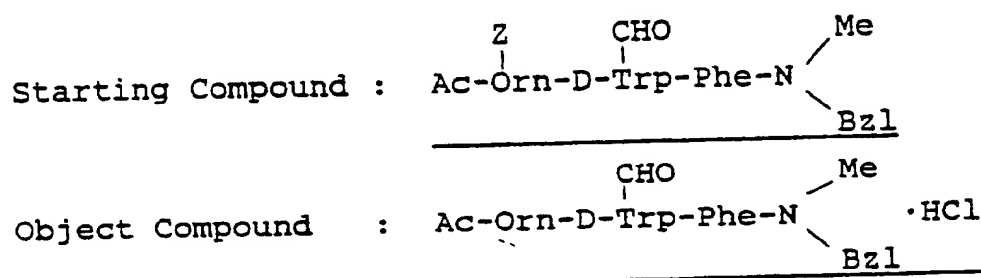
55 dissolved in a mixture of methylene chloride (10 ml) and DMF (1 ml) and ice-cooled. To the solution, triethylamine (0.41 ml) and  $\text{Ac}_2\text{O}$  (0.09 ml) were added. After stirring for an hour and twenty minutes, triethylamine (0.12 ml) and  $\text{Ac}_2\text{O}$  (0.09 ml) were added and stirred for additional half an hour. The mixture was evaporated and the residue was extracted with ethyl acetate. The organic layer was washed

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.90 (3H, d,  $J=6$ Hz), 1.33 (9H, s), 2.5-3.2 (7H, m), 2.77 (s) and 2.86 (s)(3H), 3.6-3.9 (1H, m), 3.9-4.85 (6H, m), 4.85-5.2 (1H, m), 6.75 (1H, br d,  $J=7$ Hz), 6.9-7.6 (20H, m), 7.6-7.9 (2H, m), 7.9-8.2 (2H, m), 8.80 (1H, br t,  $J=9$ Hz), 9.2 (1H, broad)  
Elemental Analysis.

	Calculated for $C_{49}H_{57}N_7O_9 \cdot 3/2H_2O$ :		
Found :	C 64.32, C 64.04,	H 6.61, H 6.41,	N 10.71 N 10.65

#### Example 34

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 28.

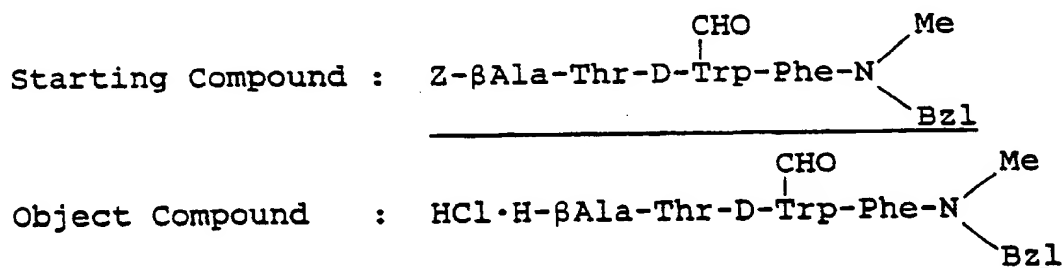


mp :  $-214^\circ\text{C}$

IR (Nujol) : 3300 (broad), 1710-1630 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.1-2.0 (4H, m), 1.80 (3H, s), 2.5-3.2 (6H, m), 2.77 (s) and 2.86 (s)(3H), 4.1-5.1 (5H, m), 6.9-7.5 (14H, m), 7.5-8.4 (6H, m), 8.70 (1H, br t,  $J=8$ Hz), 9.3 (1H, broad)

#### Example 35



$\text{Z-}\beta\text{Ala-Thr-D-Trp(CHO)-Phe-NMeBzl}$  (0.32 g) was hydrogenated with 10% palladium on carbon (0.10 g) in AcOH (10 ml). The catalyst was filtered off and the filtrate was concentrated under reduced pressure. To the residue was added 4N-HCl/DOX (0.4 ml) and evaporated. The residue was pulverized with diethyl ether, filtered, washed with diethyl ether, and dried to give

$\text{HCl}\cdot\text{H-}\beta\text{Ala-Thr-D-Trp(CHO)-Phe-NMeBzl}$  (0.26 g).

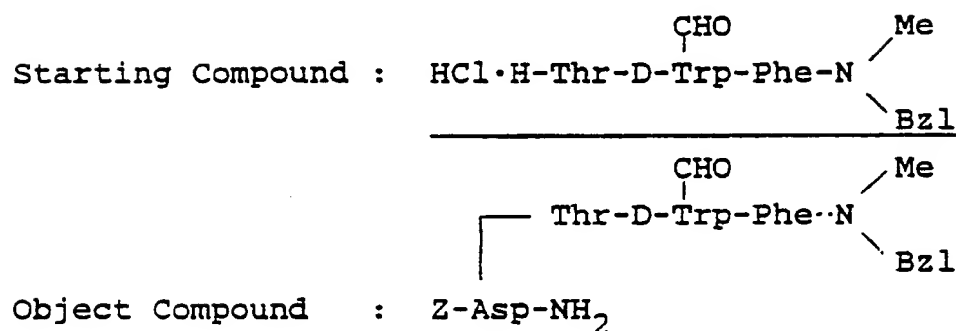
mp :  $-155^\circ\text{C}$  (dec.)

IR (Nujol) : 3300 (broad), 1640 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.82 (3H, d,  $J=6$ Hz), 2.5-3.1 (8H, m), 2.78 (s) and 2.85 (s)(3H), 3.1-5.1 (10H, m), 6.8-7.3 (11H, m), 7.3-7.7 (2H, m), 7.7-8.2 (4H, m), 8.3-8.6 (1H, m), 9.2 (1H, broad)

	Calculated for $C_{44}H_{48}N_6O_8$ :		
Found :	C 66.99, C 66.90,	H 6.13, H 6.14,	N 10.65 N 10.74

(2)



mp : 215-217° C

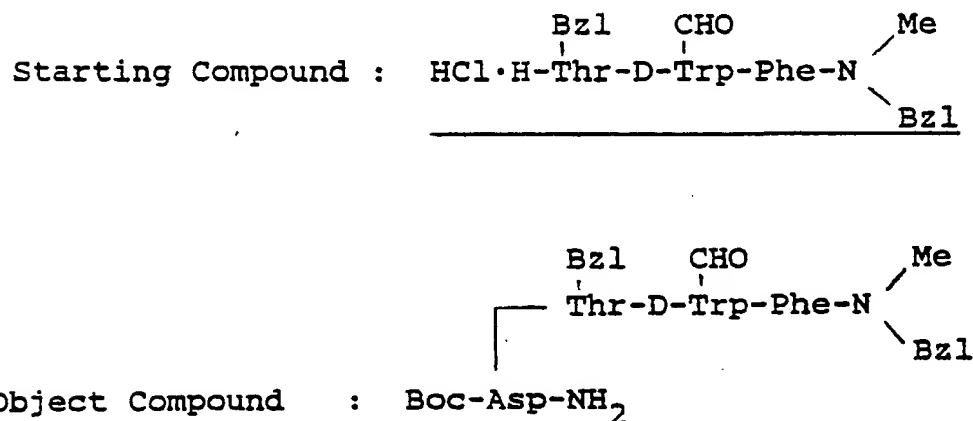
IR (Nujol) : 3300, 1705, 1695, 1650 (broad), 1550  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, t, J=6Hz), 2.5-3.2 (6H, m), 2.75 (s) and 2.84 (s)(3H), 3.6-4.0 (1H, m), 4.0-4.5 (3H, m), 4.5-5.0 (4H, m), 4.97 (2H, s), 6.9-7.6 (21H, m), 7.6-7.9 (2H, m), 7.9-8.4 (2H, m), 8.66 (1H, br t, J=9Hz), 9.2 (1H, br s)

Elemental Analysis.

	Calculated for $C_{45}H_{49}N_7O_9 \cdot H_2O$ :		
Found :	C 63.59, C 63.54,	H 6.05, H 6.02,	N 11.53 N 11.48

(3)



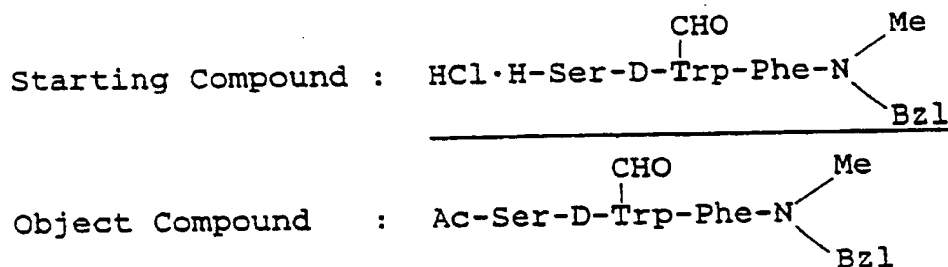
mp : -195° C (dec.)

IR (Nujol) : 3200, 1710, 1690, 1660, 1640  $cm^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.9-1.5 (4H, m), 1.77 (3H, s), 2.6-3.2 (6H, m), 2.77 (s) and 2.86 (s)(3H), 4.0-5.1 (5H, m), 4.97 (2H, s), 6.9-7.6 (19H, m), 7.6-8.0 (2H, m), 8.0-8.3 (2H, m), 8.65 (1H, br t, J = 9Hz), 9.2 (1H broad)  
Elemental Analysis.

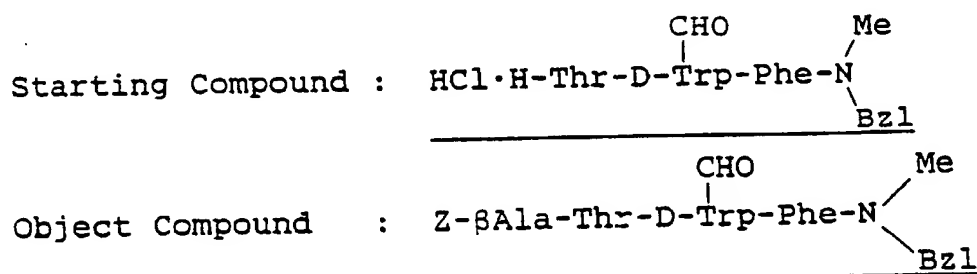
	Calculated for $C_{44}H_{48}N_6O_7 \cdot 1/2H_2O$ :		
Found :	C 67.59, C 67.73,	H 6.32, H 6.63,	N 10.75 N 10.65

(2)

mp :  $\sim 125^\circ C$  (dec.)IR (Nujol) : 3330, 1710, 1640, 1530 (broad)  $cm^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.82 (3H, s), 3.5-3.1 (4H, m), 2.77 (s) and 2.85 (s)(3H), 3.40 (2H, t, J = 6Hz), 4.0-5.1 (6H, m), 6.9-7.7 (14H, m), 7.80 (1H, d, J = 8Hz), 7.9-8.1 (2H, m), 8.62 (1H, t, J = 8Hz), 9.2 (1H, broad)Example 33

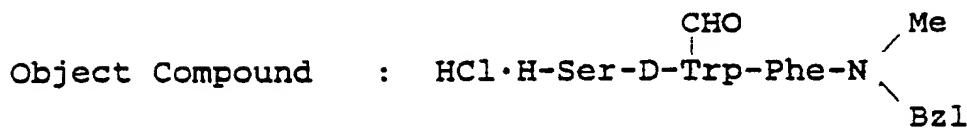
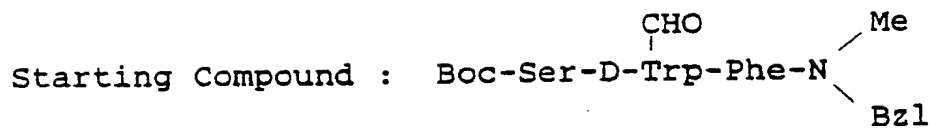
The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 29.

(1)

mp :  $\sim 177^\circ C$  (dec.)IR (Nujol) : 3300, 1710, 1690, 1640, 1535  $cm^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.75 (3H, d, J = 6Hz); 2.36 (2H, t, J = 7Hz), 2.5-3.3 (6H, m), 2.77 (s) and 2.84 (s) (s), 3.5-3.9 (1H, m), 3.9-4.2 (1H, m), 4.2-5.0 (5H, m), 4.96 (2H, s), 6.8-7.5 (18H, m), 7.5-7.8 (2H, m), 7.8-8.2 (2H, m), 8.61 (1H, t, J = 9Hz), 9.2 (1H, broad)

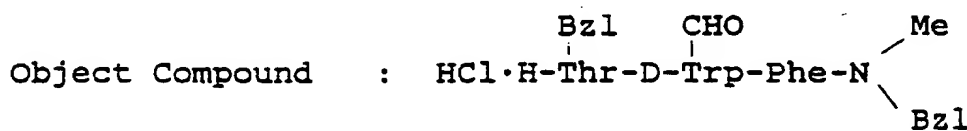
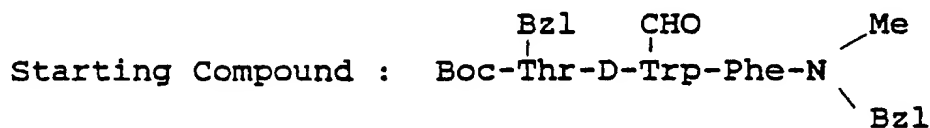
Elemental Analysis.

(3)



NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.6-3.1 (4H, m), 2.80 (s) and 2.89 (s)(3H), 3.1-3.9 (3H, m), 4.2-5.1 (4H, m), 5.3 (1H, broad), 6.9-7.7 (14H, m), 8.08 (4H, br s), 8.65 (1H, br d, J=9Hz), 8.90 (1H, br t, J=8Hz), 9.3 (1H, broad)

(4)

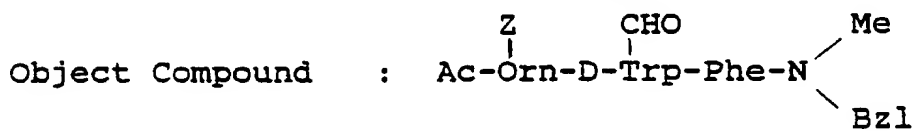
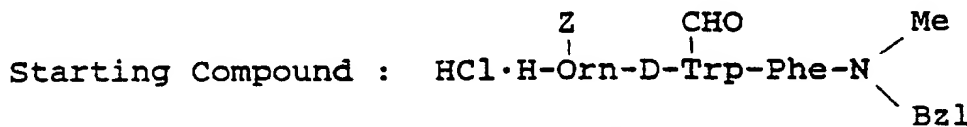


NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.84 (3H, d, J=6Hz), 2.5-3.1 (4H, m), 3.80 (s) and 2.88 (s)(3H), 3.4-5.1 (8H, m), 6.8-7.4 (17H, m), 7.60 (1H, br s), 7.65-7.85 (1H, m), 7.85-8.3 (4H, m), 8.93 (2H, m), 9.2 (1H, broad)

Example 32

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 17.

(1)



mp :  $-212^\circ \text{C}$  (dec.)

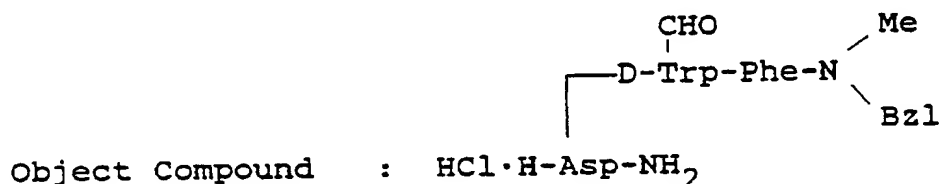
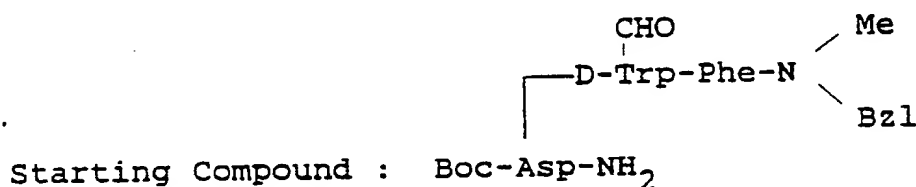
IR (Nujol) : 3300, 1710, 1700, 1640, 1540 (broad)  $\text{cm}^{-1}$

	Calculated for $C_{39}H_{47}N_5O_7 \cdot H_2O$		
	:		
Found :	C 65.44,	H 6.90,	N 9.78
	C 65.65,	H 6.66,	N 9.45

### Example 31

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 15.

(1)

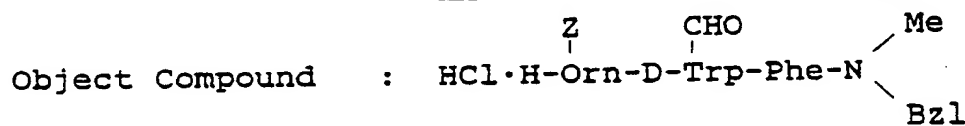
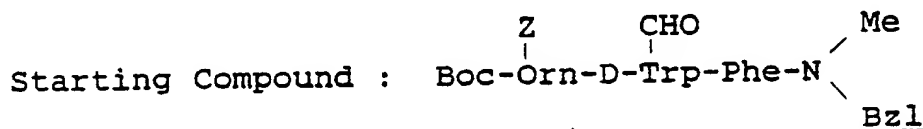


mp :  $-178^\circ \text{C}$  (dec.)

IR (Nujol) : 3250 (broad), 1700 (broad), 1640 (broad)  $\text{cm}^{-1}$

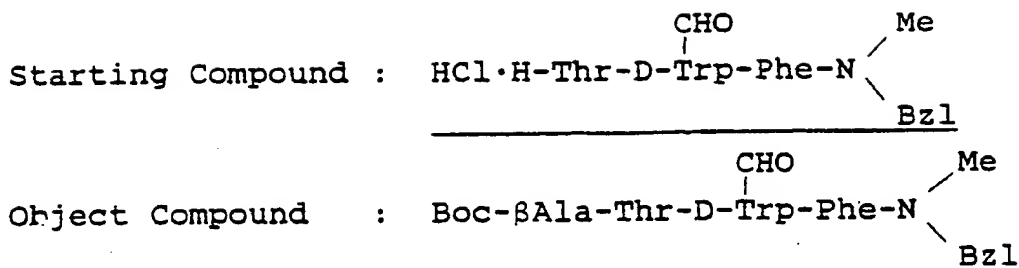
NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.5-3.1 (6H, m), 2.78 (s) and 2.86 (s)(3H), 3.8-5.1 (5H, m), 6.9-7.9 (16H, m), 8.2 (4H, br s), 8.3-8.5 (1H, m), 8.77 (1H, br t,  $J=9\text{Hz}$ ), 9.3 (1H, broad)

(2)



NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.9-1.7 (4H, m), 2.5-3.2 (6H, m), 2.78 (s) and 2.87 (s)(3H), 3.6-3.9 (1H, m), 4.1-5.1 (4H, m), 4.96 (2H, s), 6.9-7.3 (18H, m), 7.3-7.6 (1H, m), 7.6-7.8 (1H, m), 8.16 (4H, br s), 8.6-9.0 (2H, m), 9.3 (1H, broad),





Boc- $\beta$ Ala-OH (0.19 g), HCl·H-Thr-D-Trp(CHO)-Phe-NMeBzl (0.62 g) and HOBT (0.14 g) were dissolved in DMF (10 ml). To this solution was added WSC (0.18 ml) under ice cooling and the mixture was stirred for four hours at room temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with water, 2% sodium hydrogencarbonate solution, water, 2% hydrochloric acid, water and saturated sodium chloride solution, and dried over magnesium sulfate. The evaporated residue was crystallized from a mixed solvent of ethanol and water. Filtration and drying gave Boc- $\beta$ Ala-Thr-D-Trp-(CHO)-Phe-NMeBzl (0.66 g).

mp : 182-192° C (dec.)

IR (Nujol) : 3430, 3350, 3300, 1705, 1690, 1640, 1530  $\text{cm}^{-1}$

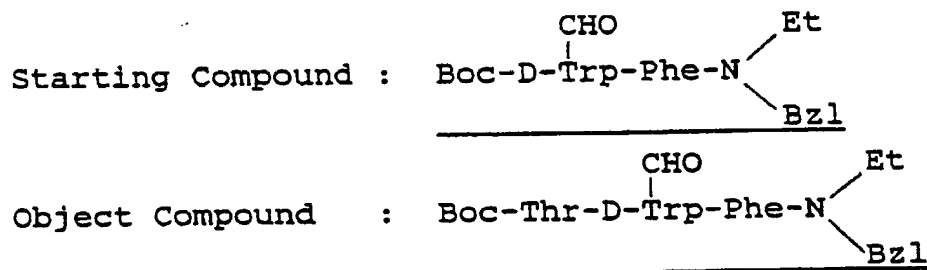
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, t, J=6Hz), 1.35 (9H, s), 2.33 (2H, t, J=7Hz), 3.5-3.3 (4H, m), 2.77 (s) and 2.84 (s)(3H), 3.07 (2H, t, J=7Hz), 3.6-3.9 (1H, m), 3.9-4.3 (1H, m), 4.3-5.2 (5H, m), 6.6 (1H, br s), 6.9-7.8 (15H, m), 7.8-8.3 (2H m), 8.60 (1H, br t, J=9Hz), 9.2 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{41}\text{H}_{50}\text{N}_6\text{O}_8$ :		
Found :	C 65.24, C 65.06,	H 6.68, H 6.70,	N 11.13 N 11.16

### Example 30

The following object compound was obtained from the corresponding starting compound according to similar manners to those of Example 2 and Example 22, successively.

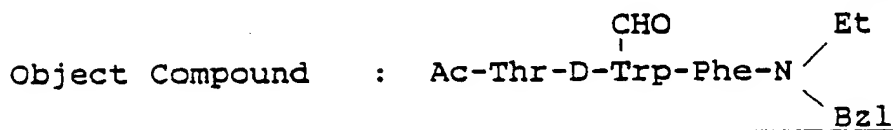


mp : 90-94° C

IR (Nujol) : 3320, 1710, 1635 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.7-1.1 (6H, m), 1.33 (9H, s), 2.5-3.4 (6H, m), 3.6-4.0 (2H, m), 4.2-5.2 (5H, m), 6.27 (1H, br d, J=9Hz), 6.9-7.8 (14H, m), 7.8-8.3 (2H, m), 8.66 (1H, br d, J=9Hz), 9.2 (1H, broad)

Elemental Analysis.



mp : 187-189 °C

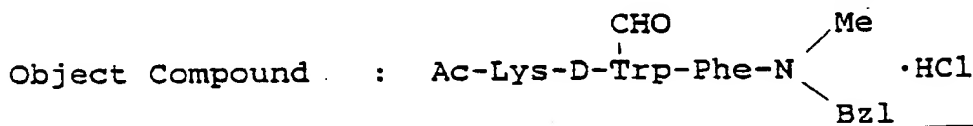
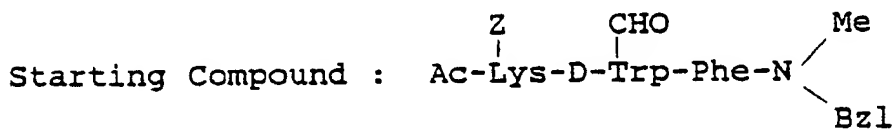
IR (Nujol) : 3510, 3340, 3300, 1710, 1660, 1550 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.78 (3H, d,  $J=6\text{Hz}$ ), 0.96 (3H, t,  $J=7\text{Hz}$ ), 1.85 (3H, s), 2.6-3.1 (4H, m), 3.1-3.5 (2H, m), 3.6-3.95 (1H, m), 4.0-4.3 (1H, m), 4.35-5.15 (5H, m), 7.0-7.8 (15H, m), 7.9-8.3 (2H, m), 8.62 (1H, br d,  $J=9\text{Hz}$ ), 9.3 (1H, broad)

Elemental Analysis.

Calculated for $\text{C}_{36}\text{H}_{41}\text{N}_5\text{O}_6 \cdot 1/2\text{H}_2\text{O}$ :			
Found :	C 66.65, C 66.35,	H 6.53, H 6.21,	N 10.80 N 10.79

#### Example 28

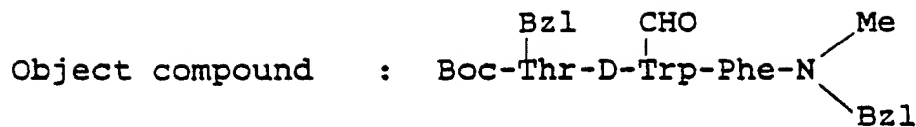


Ac-Lys(Z)-D-Trp(CHO)-Phe-NMeBzL (0.54 g) was hydrogenated in AcOH (20 ml) with 10% palladium on carbon (0.10 g). The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in methanol. To the solution was added 4N-HCl/DOx (0.35 ml) and evaporated. The residue was dissolved in ethanol and the solution was treated with activated charcoal. The charcoal was filtered off and the filtrate was concentrated under reduced pressure. The residue was pulverized with diisopropyl ether, filtered, washed with diisopropyl ether and dried to give Ac-Lys-D-Trp(CHO)-Phe-NMeBzL·HCl (0.45 g).

IR (Nujol) : 3450 (broad), 1640 (broad), 1540 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.8-1.8 (6H, m), 1.77 (3H, s), 2.5-3.1 (6H, m), 2.77 (s) and 2.86 (s)(3H), 3.3-4.0 (3H, broad), 4.0-5.2 (5H, m), 6.9-7.6 (11H, m), 7.6-8.4 (6H, m), 8.5-8.8 (1H, m), 9.4 (1H, broad)

#### Example 29



5

mp : 185-186 °C

IR (Nujol) : 3350, 3300, 1695, 1645, 1630 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.83 (3H, d, J=6Hz), 1.34 (9H, s), 2.5-3.1 (4H, m), 2.76 (s) and 2.85 (s) (3H), 3.4-3.7 (1H, m), 3.8-5.2 (7H, m), 6.17 (1H, br d, J=7Hz), 6.9-7.6 (18H, m), 7.6-7.8 (1H, m), 7.8-8.3 (2H, m), 8.75 (1H, br t, J=9Hz), 9.2 (1H, broad)

Elemental Analysis.

15

	Calculated for C <sub>45</sub> H <sub>51</sub> N <sub>5</sub> O <sub>7</sub> * 1/2H <sub>2</sub> O:		
Found :	C 69.03, C 68.99,	H 6.69, H 6.40,	N 8.94 N 8.97

20

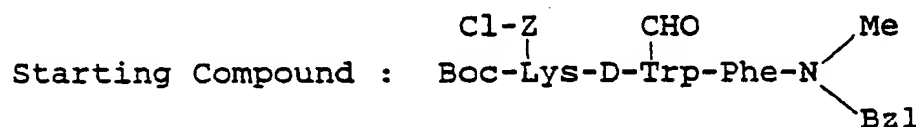
Example 27

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 23.

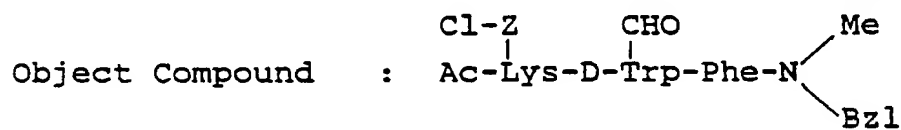
25

(1)

30



35



40

mp : 190-192 °C

IR (Nujol) : 3300, 1710, 1690, 1640, 1545 (board), cm<sup>-1</sup>m

NMR (DMSO-d<sub>6</sub>, δ) : 0.7-1.5 (6H, m), 1.70 (3H, s), 2.5-3.1 (6H, m), 2.70 (s) and 2.80 (s) (3H), 3.9-5.1 (5H, m), 4.98 (2H, s), 6.9-7.5 (18H, m), 7.5-7.9 (2H, m), 7.9-8.3 (2H, m), 8.57 (1H, br t, J=9Hz), 9.3 (1H, broad)

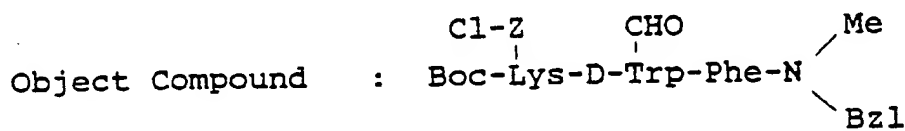
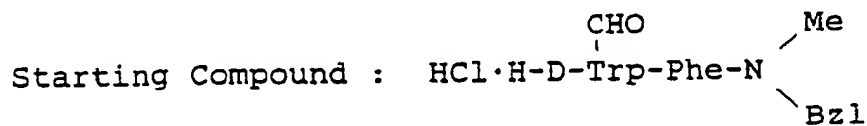
45

Elemental Analysis.

50

	Calculated for C <sub>45</sub> H <sub>49</sub> ClN <sub>5</sub> O <sub>7</sub> :		
Found :	C 65.80, C 65.72,	H 6.01, H 6.00,	N 10.23 N 10.18

55 (2)



mp :  $-124^{\circ}\text{C}$  (dec.)

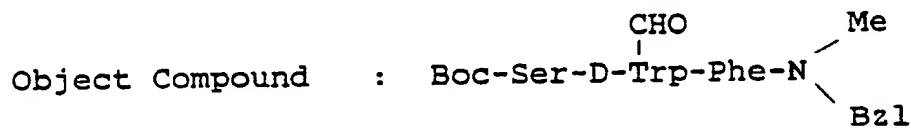
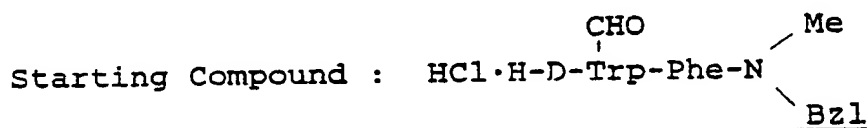
IR (Nujol) : 3300, 1690 (board), 1645, 1530 (broad)  $\text{cm}^{-1}$

MNR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.8-1.5 (6H, m), 1.30 (9H, s), 2.5-3.1 (6H, m), 2.68 (s) and 2.76 (s) (3H), 3.6-4.0 (1H, m), 4.1-5.1 (4H, m), 4.95 (2H, s), 6.55 (1H broad), 6.8-7.8 (19H, m), 7.8-8.3 (2H, m), 8.3-8.8 (1H, m), 9.25 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{48}\text{H}_{55}\text{ClN}_5\text{O}_8$ :		
	C 65.56,	H 6.30,	N 9.56
Found :	C 65.61,	H 6.29,	N 9.52

(5)

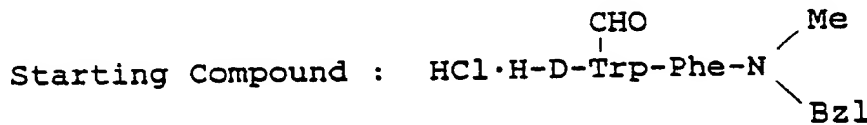


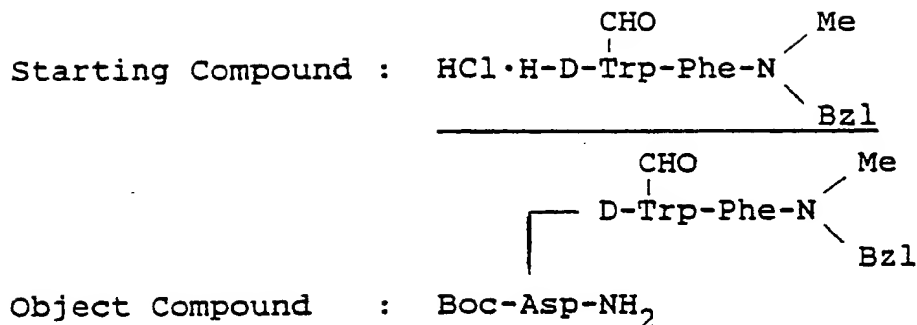
mp :  $-112^{\circ}\text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1640  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 1.34 (9H, s), 3.5-3.1 (4H, m), 2.77 (s) and 2.9 (s)(3H), 3.42 (2H, br t,  $J=6\text{Hz}$ ), 3.7-5.1 (6H, m), 6.51 (1H, br d,  $J=7\text{Hz}$ ), 6.9-7.7 (14H, m), 7.8-8.2 (2H, m), 8.64 (1H, br t,  $J=8\text{Hz}$ ), 9.15 (1H, broad)

(6)





mp : 213-216 °C

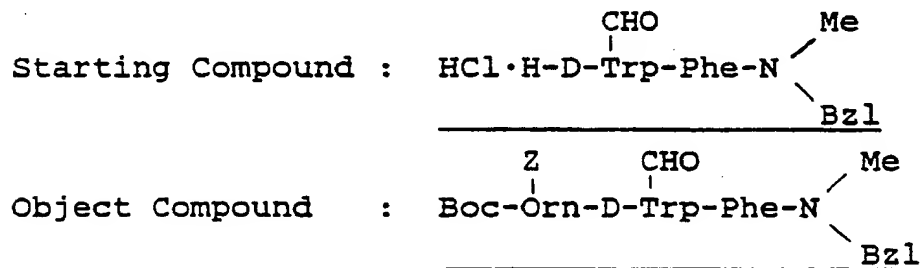
IR (Nujol) : 3400, 3340, 3300, 3230, 1715, 1670, 1640, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 2.3-2.5 (2H, m), 2.6-3.2 (4H, m), 2.76 (s) and 2.83 (s) (3H), 4.0-5.1 (5H, m), 6.6-7.7 (17H, m), 7.8-8.3 (2H, m), 8.4-8.8 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{38}\text{H}_{44}\text{N}_6\text{O}_7$ :		
Found :	C 65.50, C 65.15,	H 6.36, H 6.28,	N 12.06 N 11.98

(3)



mp : -171 °C

IR (Nujol) : 3330, 3300, 1710, 1695, 1645, 1530 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.9-1.5 (4H, m), 1.33 (9H, s), 2.5-3.1 (6H, m), 2.77 (s) and 2.85(s) (3H), 3.7-4.0 (1H, m), 4.1-5.1 (4H, m), 4.97 (2H, s), 6.63 (1H, br d,  $J = 7\text{Hz}$ ), 6.9-7.5 (19H, m), 7.5-7.8 (1H, m), 7.8-8.3 (2H, m), 8.5-8.8 (1H, m), 9.2 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{47}\text{H}_{54}\text{N}_6\text{O}_8$ :		
Found :	C 67.93, C 67.63,	H 6.55, H 6.76,	N 10.11 N 10.02

(4)

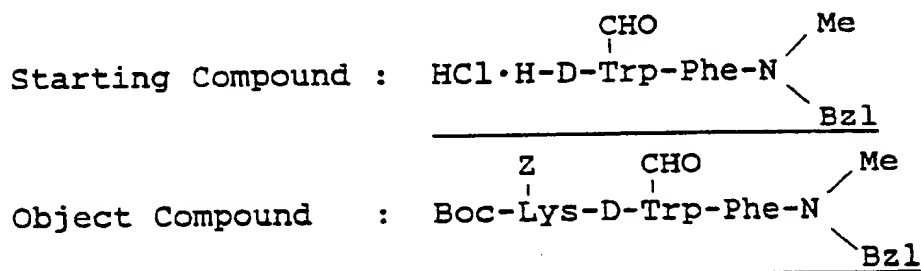
## Elemental Analysis.

	Calculated for $C_{39}H_{42}N_6O_7 \cdot 1/2H_2O$ :		
Found :	C 65.44, C 65.59,	H 6.05, H 5.90,	N 11.74 N 11.84

Example 26

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 13.

(1)



mp : 74-80 °C

IR (Nujol) : 3300, 1710, 1640  $cm^{-1}$ 

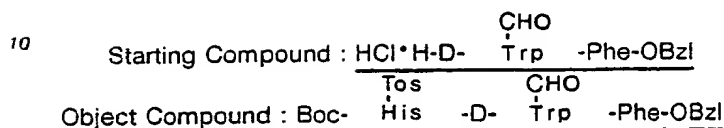
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.8-1.5 (6H, m), 1.30 (9H, s), 2.5-3.1 (6H, m), 2.77 (s) and 2.80 (s) (3H), 3.6-4.0 (4H, m), 4.2-5.0 (4H, m), 4.97 (2H, s), 6.6 (1H, broad), 6.9-7.5 (19H, m), 7.5-7.8 (1H, m), 7.8-8.3 (2H, m), 8.45-8.85 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{48}H_{56}N_6O_8 \cdot 1/2H_2O$ :		
Found :	C 67.51, C 67.32,	H 6.73, H 6.47,	N 9.84 N 9.69

(2)

	Calculated for $C_{45}H_{50}N_6O_7$ :		
Found :	C 68.68, C 68.33,	H 6.40, H 6.22,	N 10.68 N 10.53

Example 24

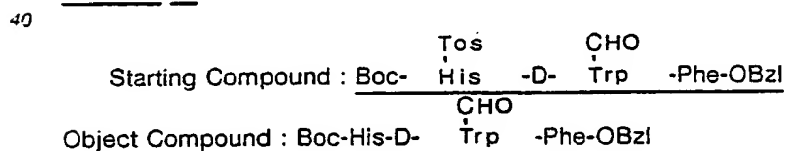
15 To a solution of Boc-His(Tos)-OH (0.81 g) in methylene chloride (10 ml) were added NMM (0.22 ml) and isobutyl chloroformate (0.26 ml) successively at  $-15^\circ\text{C}$ , and the mixture was stirred for ten minutes. On the other hand, a solution of  $\text{HCl} \cdot \text{H-D-Trp(CHO)-Phe-OBzl}$  (1.00 g) in DMF (20 ml). This solution was added to the above mentioned mixture and stirred for two hours at  $-30^\circ\text{C}$ . After evaporation and extraction with ethyl acetate, the organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride solution, and dried over magnesium sulfate.

20 After evaporation, the residue was subjected to column chromatography on silica gel (100 g) and eluted with a mixture of chloroform and methanol (100:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with n-hexane, filtered, washed with N-hexane and dried to give Boc-His(Tos)-d-Trp(CHO-Phe-OBzl) (1.42 g).

25 mp :  $107-111^\circ\text{C}$   
 IR (Nujol) : 3300, 1700 (broad),  $1645\text{ cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 2.37 (3H, s), 2.4-3.1 (6H, m), 4.0-4.4 (1H, m), 4.4-4.9 (2H, m), 5.14 (2H, s), 6.7-6.9 (1H, m), 7.1-7.7 (6H, m), 7.25 (5H, s), 7.37 (5H, s), 7.50 (2H, d,  $J=8\text{Hz}$ ), 7.94 (2H, d,  $J=8\text{Hz}$ ), 7.9-8.3 (1H, m), 8.32 (1H, s), 8.75 (1H, br d,  $J=7\text{Hz}$ ), 9.3 (1H, broad)

30 Elemental Analysis.

	Calculated for $C_{45}H_{48}N_6O_9S$ :		
Found :	C 64.17, C 64.00,	H 5.62, H 5.76,	N 9.76 N 9.61

Example 25

45 To a solution of Boc-His(Tos)-D-Trp(CHO)-Phe-OBzl (1.16 g) in DMF (35 ml) was added pyridinium chloride (1.6 g) at room temperature. After stirring for one and half an hour, additional pyridinium chloride (0.4 g) was added and the mixture was stirred for additional 50 minutes. After evaporation, the residue was solidified with water, filtered, washed with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and dried. The powder was subjected to column chromatography on silica gel (100 g) and eluted with a mixture of chloroform and methanol (20:1). The fractions containing the object compound were combined and evaporated. The residue was dissolved in ethanol and reprecipitated with water, filtered and dried to give Boc-His-D-Trp(CHO)-Phe-OBzl (0.70 g).

50 mp :  $112-115^\circ\text{C}$   
 IR (Nujol) : 3300, 1710 (broad),  $1640\text{ cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.25 (9H, s), 2.5-3.1 (6H, m), 3.8-4.3 (1H, m), 4.3-4.8 (2H, m), 5.03 (2H, s), 6.5-6.7 (1H, m), 6.54 (1H, s), 7.0-7.6 (4H, m), 7.13 (5H, s), 7.27 (5H, s), 7.44 (1H, s), 7.8-8.3 (2H, m), 8.66 (1H, br d,  $J=9\text{Hz}$ ), 9.2 (1H, broad)

To a solution of 2HCl·H-D-Trp(CHO)-Phe-OCH<sub>2</sub>Py(2) (0.74 g), BOC-Gln-OH (0.30 g) and HOBT (0.16 g) in DMF (15 ml) were added N,N-diisopropyl-N-ethylamine (0.21 ml) and WSC (0.22 ml) successively under ice cooling, and the mixture was stirred for two hours at room temperature. After evaporation, the residue was pulverized with water, filtered, and washed with water, 2% sodium hydrogencarbonate solution and water. The solids were dissolved in DMF and reprecipitated with ethyl acetate, filtered and dried to give Boc-Gln-D-Trp(CHO)-Phe-OCH<sub>2</sub>Py(2) (0.66 g).

mp : 166-170 °C

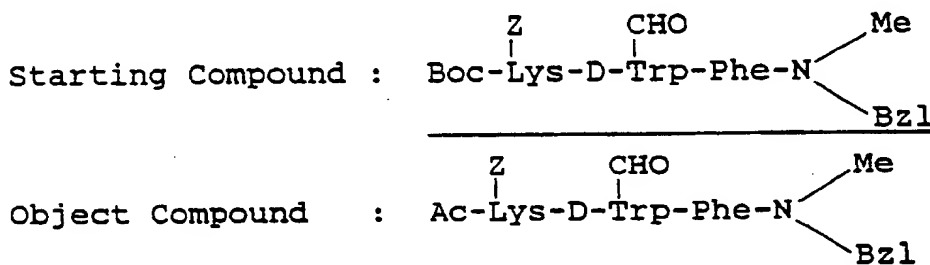
IR (Nujol) : 3300, 1740, 1710, 1690, 1650 (broad), 1525 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.31 (9H, s), 1.4-2.1 (4H, m), 2.6-3.2 (4H, m), 3.7-4.1 (1H, m), 4.4-4.9 (2H, m), 5.21 (2H, s), 6.6-6.9 (2H, m), 7.0-8.3 (15H, m), 8.5-8.6 (1H, m), 8.6-8.8 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for C <sub>37</sub> H <sub>42</sub> N <sub>6</sub> O <sub>8</sub> :		
	C 63.60,	H 6.06,	N 12.03
Found :	C 63.29,	H 6.13,	N 12.00

### Example 23



To a solution of Boc-Lys(Z)-D-Trp(CHO)-Phe-NMeBzl (1.04 g) in methylene chloride (10 ml) was added 4N-HCl/DOX (10 ml) under ice-cooling. The mixture was stirred for an hour at room temperature. After evaporation, the residue was pulverized with diisopropyl ether, filtered, washed with diisopropyl ether and dried. The obtained HCl·H-Lys(Z)-D-Trp(CHO)-Phe-NMeBzl (0.94 g) was dissolved in methylene chloride (15 ml) and cooled in an ice-bath. To the solution were added triethylamine (0.34 ml) and Ac<sub>2</sub>O (0.11 ml) and the mixture was stirred for an hour at the same temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with water, 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride, and then dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (50 g) and eluted with a mixture of chloroform and methanol (50 ml). The fractions containing the object compound were combined and evaporated. The residue was pulverized with n-hexane, filtered, washed with n-hexane and dried to give Ac-Lys(Z)-D-Trp(CHO)-Phe-NMeBzl (0.82 g).

mp : ~174 °C (dec.)

IR (Nujol) : 3300, 1710, 1690, 1640, 1540 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.8-1.5 (6H, m), 1.78 (3H, s), 2.6-3.2 (6H, m), 2.78 (s) and 2.87 (s) (3H), 4.0-5.2 (5H, m), 4.98 (2H, s), 6.9-7.6 (19H, m), 7.6-7.9 (2H, m), 7.9-8.3 (2H, m), 8.64 (1H, br t, J=9Hz), 9.3 (1H, broad)

Elemental Analysis.



Example 20

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 11.

(1)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Boc-D-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$   
 mp : 170-172 °C

IR (Nujol) : 3300, 1720, 1660, 1640, 1550, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.32 (9H, s), 1.5-2.2 (4H, m), 2.6-3.2 (4H, m), 3.6-4.1 (1H, m), 4.4-4.9 (2H, m), 5.12 (2H, s), 6.6-7.0 (2H, m), 7.0-7.7 (5H, m), 7.25 (5H, s), 7.36 (5H, s), 7.90 (1H, br d, J=9Hz), 8.0-8.3 (1H, m), 8.76 (1H, br d, J=8Hz), 9.2 (1H, broad)

(2)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Boc-} \begin{array}{c} \text{Troc} \\ | \\ \text{Lys} \end{array} \text{-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$   
 mp : 160-162 °C

IR(Nujol) : 3350, 3300, 1720, 1710, 1690, 1645, 1545, 1520  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.8-1.5 (6H, m), 1.32 (9H, s), 2.5-3.1 (6H, m), 3.7-4.0 (1H, m), 4.4-4.8 (2H, m), 4.81 (2H, s), 5.15 (2H, s), 6.6-6.8 (1H, m), 7.1-7.8 (5H, m), 7.27 (5H, s), 7.39 (5H, s), 7.9-8.4 (2H, m), 8.5-8.8 (1H, m), 9.3 (1H, broad)

Example 21

Starting Compound :  $\text{Boc-} \begin{array}{c} \text{Troc} \\ | \\ \text{Lys} \end{array} \text{-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Boc-Lys-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl} \cdot \text{AcOH}$

To a solution of Boc-Lys(Troc)-D-Trp(CHO)-Phe-OBzl (0.94 g) in 90%AcOH (20 ml) was added zinc (0.94 g) and the mixture was stirred overnight at room temperature. Insoluble materials were filtered off and the filtrate was evaporated. The residue was subjected to column chromatography on silica gel (50 g) and eluted successively with a mixture of chloroform and methanol (10:1) and then a mixture of chloroform, methanol and AcOH (8:1:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with n-hexane, filtered, washed with n-hexane, and dried to give Boc-Lys-D-Trp(CHO)-Phe-OBzl  $\cdot$  AcOH (0.42 g).

mp :  $\sim 175^\circ \text{C}$  (dec.)

IR (Nujol) : 3320, 1690 (broad), 1640, 1550, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.8-1.5 (6H, m), 1.32 (9H, s), 1.87 (3H, s), 2.5-3.2 (6H, m), 3.8-4.1 (1H, m), 4.3-5.5 (5H, m), 5.12 (2H, s), 6.6-6.8 (1H, m), 6.8-7.1 (1H, m), 7.1-7.8 (3H, m), 7.23 (5H, s), 7.33 (5H, s), 7.9-8.3 (2H, m), 8.6-8.9 (1H, m), 9.3 (1H, broad)

Example 22

Starting Compound :  $2\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(2)}$

Object Compound :  $\text{Boc-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(2)}$

(2)

Starting Compound :  $\text{HCl} \cdot \text{H} - \text{Glu}(\text{NMe}_2) - \text{D} - \text{Trp}(\text{CHO}) - \text{Phe} - \text{N} \begin{matrix} \text{Me} \\ \text{Bzl} \end{matrix}$

Object Compound :  $\text{Ac} - \text{Glu}(\text{NMe}_2) - \text{D} - \text{Trp}(\text{CHO}) - \text{Phe} - \text{N} \begin{matrix} \text{Me} \\ \text{Bzl} \end{matrix}$

mp :  $-120^\circ \text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1640 (broad), 1545 (sh), 1530, 1490  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.3-2.1 (4H, m) 1.79 (3H, s), 2.5-3.2 (4H, m), 2.63 (3H, s), 2.73 (3H, s), 2.82 (s) and 2.90 (s) (3H), 4.0-5.2 (5H, m), 6.9-7.6 (13H, m), 7.6-8.3 (4H, m), 8.5-8.9 (1H, m), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{38}\text{H}_{44}\text{N}_6\text{O}_6 \cdot 1/2\text{H}_2\text{O}$ :		
Found :	C 66.17, C 65.99,	H 6.58, H 6.65,	N 12.18 N 11.94

#### Example 19

The following object compound was obtained from the corresponding starting compound according to similar manners to those of Example 4 and Example 13, successively.

Starting Compound :  $\text{Boc} - \text{D} - \text{Trp}(\text{Tos}) - \text{Phe} - \text{N} \begin{matrix} \text{Me} \\ \text{Bzl} \end{matrix}$

Object Compound :  $\text{Boc} - \text{Thr} - \text{D} - \text{Trp}(\text{Tos}) - \text{Phe} - \text{N} \begin{matrix} \text{Me} \\ \text{Bzl} \end{matrix}$

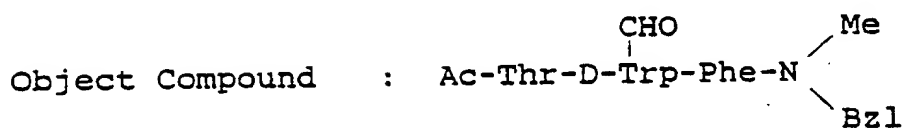
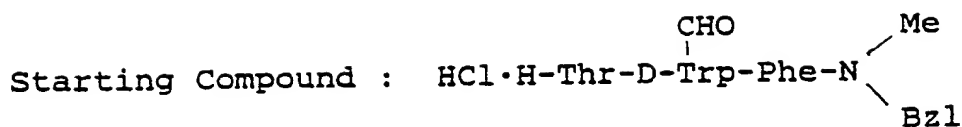
mp :  $95-96^\circ \text{C}$

IR (Nujol) : 3350, 1695, 1655, 1620  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.86 (3H, d,  $J=6\text{Hz}$ ), 1.38 (9H, s), 2.27 (3H, s), 2.72 and 2.80 (3H, s), 2.6-3.2 (4H, m), 3.7-4.05 (2H, m), 4.2-5.1 (6H, m), 6.33 (1H, d,  $J=6\text{Hz}$ ), 6.95-7.9 (19H, m), 8.0-8.2 (1H, m), 8.5-8.75 (1H, m)

Elemental Analysis.

	Calculated for $\text{C}_{44}\text{H}_{51}\text{N}_5\text{O}_8\text{S}_1$ :		
Found :	C 65.25, C 64.97,	H 6.35, H 6.39,	N 8.65 N 8.51



To a solution of  $\text{HCl} \cdot \text{H-Thr-D-Trp(CHO)-Phe-NMeBzl}$  (2.29 g) in methylene chloride (30 ml), were added triethylamine (747 mg) and  $\text{Ac}_2\text{O}$  (377 mg) at  $-20^\circ\text{C}$ . The reaction mixture was stirred for 45 minutes at the same temperature, and washed successively with water, diluted sodium hydrogencarbonate solution, water, 0.5N hydrochloric acid, and sodium chloride solution and dried over magnesium sulfate. After concentration, the residue was dissolved in 65% aqueous ethanol (45 ml) under heating, and the solution was left standing overnight at room temperature. The resulting needles were filtered, washed with 65% aqueous ethanol, and dried to give  $\text{Ac-Thr-D-Trp(CHO)-Phe-NMeBzl}$  (1.92 g).

mp :  $179.5-180.5^\circ\text{C}$

IR (Nujol) : 3450 (sh), 3260, 1720 (sh), 1698, 1660 (sh), 1645-1620 (broad),  $1550\text{ cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 1.87 (3H, s), 2.80 (s) and 2.87 (s) (3H), 2.6-3.2 (4H, m), 3.6-3.9 (1H, m), 3.95-4.3 (1H, m), 4.3-5.2 (5H, m), 6.95-7.8 (15H, m), 7.8-8.3 (2H, m), 8.5-8.75 (1H, m), 9.0-9.7 (1H, br s)

Elemental Analysis.

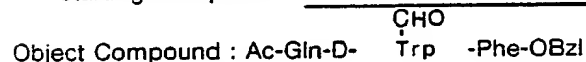
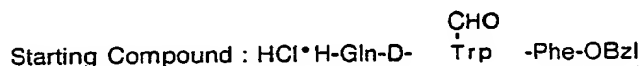
	Calculated for $\text{C}_{35}\text{H}_{39}\text{N}_5\text{O}_6 \cdot \text{H}_2\text{O}$ :		
	C 65.30,	H 6.42,	N 10.88
Found :	C 65.54,	H 6.41,	N 10.99

$[\alpha]_D^{25} + 20.03^\circ$  (c 1.078, DMF)

#### Example 18

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 17.

(1)



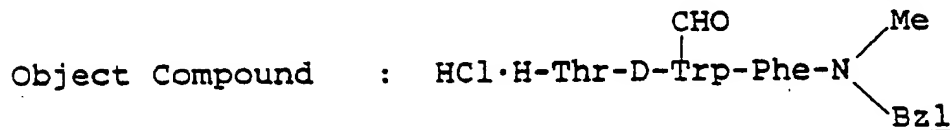
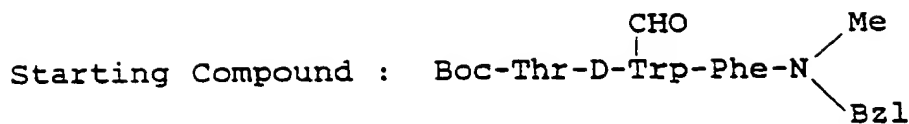
mp :  $\sim 233^\circ\text{C}$  (dec.)

IR (Nujol) : 3420, 3290, 3220 (sh), 1725, 1710, 1655, 1640, 1630 (sh),  $1545\text{ cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 1.4-2.1 (4H, m), 1.80 (3H, s), 2.6-3.2 (4H, m), 4.0-4.4 (1H, m), 4.4-4.8 (2H, m), 5.13 (2H, s), 6.70 (1H, br s), 7.0-7.8 (5H, m), 7.23 (5H, s), 7.35 (5H, s), 8.00 (1H, br d,  $J=9\text{Hz}$ ), 8.21 (2H, br d,  $J=9\text{Hz}$ ), 8.68 (1H, br d,  $J=8\text{Hz}$ ), 9.30 (1H, br d)

Elemental Analysis.

	Calculated for $\text{C}_{35}\text{H}_{37}\text{N}_5\text{O}_7$ :		
	C 65.72,	H 5.83,	N 10.95
Found :	C 65.32,	H 5.78,	N 10.95



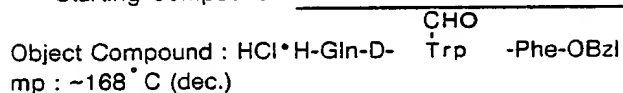
Boc-Thr-D-Trp(CHO-Phe-NMeBzl) (2.54 g) and anisole (2.5 ml) were dissolved in methylene chloride (25 ml) and ice-cooled. To this solution was added 4N-HCl/DOX (25 ml). The reaction mixture was stirred for an hour at room temperature. After evaporation, the residue was triturated with diisopropyl ether, filtered, washed with diisopropyl ether, and dried to give HCl·H-Thr-D-Trp(CHO)-Phe-NMeBzl (2.30 g).

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.77 (3H, tr, H=6Hz), 2.80 (s), and 2.88 (s) (3H), 2.6-3.0 (4H, m), 3.5-3.8 (2H, m), 4.15-5.1 (5H, m), 6.95-7.4 (14H, m), 7.4-7.8 (2H, m), 8.10 (3H, br s), 8.6-9.0 (2H, m), 9.1-9.7 (1H, br)

#### Example 16

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 15.

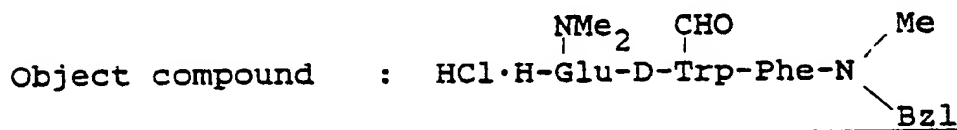
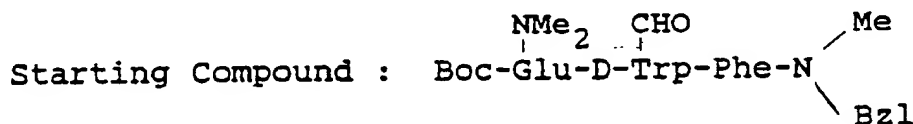
(1)



IR (Nujol) : 3200 (broad), 1735 (sh), 1710 (sh), 1690 (sh), 1675 (sh), 1660, 1605, 1530 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.5-2.2 (4H, m), 2.6-3.3 (4H, m), 3.6-4.0 (1H, m), 4.4-5.0 (2H, m), 5.14 (2H, s), 6.90 (1H, br s), 7.0-7.8 (5H, m), 7.27 (5H, s), 7.38 (5H, s), 8.33 (4H, br s), 8.7-9.2 (2H, m), 9.3 (1H, br s)

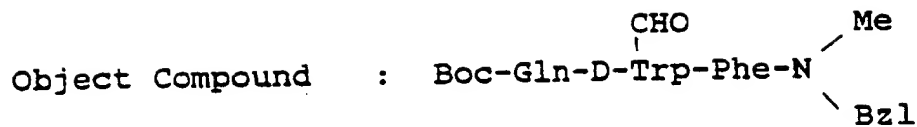
(2)



IR (Nujol) : 3400 (sh), 3200 (broad), 1710 (broad), 1630, 1490  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.4-2.3 (4H, m), 2.5-3.2 (4H, m), 2.57 (3H, s), 2.77 (3H, s), 2.85 (s) and 2.96 (s) (3H), 3.6-4.0 (1H, m), 4.2-5.2 (4H, m), 7.0-7.7 (14H, m), 7.7-8.0 (1H, m), 8.22 (3H, br s), 8.6-9.6 (3H, m)

#### Example 17



mp : 197-199 °C

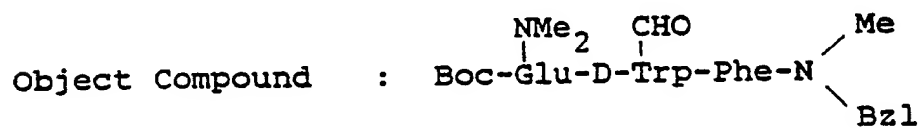
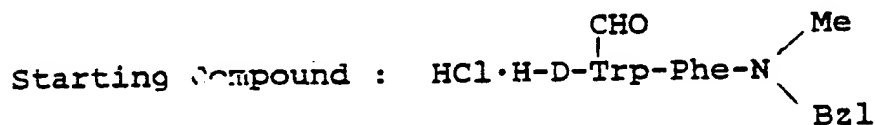
IR (Nujol) : 3340, 3350 (sh), 3300, 3240 (sh), 1715, 1690, 1665, 1650, 1635, 1550, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.33 (9H, s), 1.5-2.2 (4H, m), 2.6-3.2 (4H, m), 2.79 (s) and 2.87 (s) (3H), 3.7-4.2 (1H, m), 4.2-5.3 (4H, m), 6.7 (2H, br s), 7.0-7.6 (14H, m), 7.6-7.9 (1H, m), 7.9-8.4 (2H, m), 8.7 (1H, br s), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for C <sub>39</sub> H <sub>45</sub> N <sub>6</sub> O <sub>7</sub> :		
	C 65.90,	H 6.52,	N 11.82
Found :	C 65.86,	H 6.41,	N 11.86

(2)



mp : ~110 °C (dec.)

IR (Nujol) : 3300, 1710, 1635, 1525 (sh), 1510 (sh), 1490 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.33 (9H, s), 1.3-2.1 (4H, m), 2.6-3.2 (4H, m), 2.69 (3H, s), 2.77 (3H, s), 2.82 (s) and 2.91 (s)(3H), 3.8-4.1 (1H, m), 4.2-5.2 (4H, m), 6.77 (1H, br d, J=6Hz), 7.0-7.7 (13H, m), 7.7-7.9 (1H, m), 7.9-8.3 (2H, m), 8.5-8.9 (1H, m), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for C <sub>41</sub> H <sub>50</sub> N <sub>6</sub> O <sub>7</sub> :		
	C 66.65,	H 6.82,	N 11.37
Found :	C 66.78,	H 7.12,	N 10.92

#### Example 15

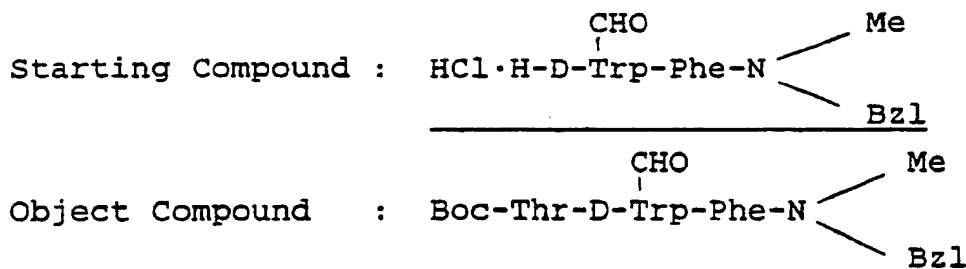
IR (Nujol) : 3420, 3340, 3300, 3240, 1735, 1690, 1665, 1640, 1620, 1540, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 1.4-2.2 (4H, m), 2.6-3.2 (4H, m), 3.7-4.2 (1H, m), 4.3-4.8 (2H, m), 5.09 (2H, s), 6.5-7.6 (19H, m), 7.90 (1H, br d,  $J=8\text{Hz}$ ), 8.51 (1H, br d,  $J=9\text{Hz}$ )

Elemental Analysis.

	Calculated for $\text{C}_{37}\text{H}_{43}\text{N}_5\text{O}_7$ :		
Found :	C 66.35, C 66.37,	H 6.47, H 6.39,	N 10.46 N 10.41

### Example 13



Boc-Thr-OH (1.23 g),  $\text{HCl} \cdot \text{H-D-Trp(CHO)-Phe-NMeBzl}$  (3.0 g) and HOBT (0.757 g) were dissolved in DMF (40 ml). To this solution was added WSC (887 mg) under ice cooling and the mixture was stirred for 1.5 hours at the same temperature and overnight at room temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with water, diluted sodium hydrogencarbonate solution, water, 0.5N hydrochloric acid, and sodium chloride solution and dried over magnesium sulfate. The evaporated residue was crystallized from a mixed solvent of ethyl acetate and diisopropyl ether (1:1) (10 ml) with seeding and the crystals were washed out by addition of diisopropyl ether (30 ml) and dried to give Boc-Thr-D-Trp(CHO)-Phe-NMeBzl (3.64 g).

mp : 104.5-111 °C (dec.)

IR (Nujol) : 3360, 3220, 3070, 1718, 1690, 1668, 1650, 1626, 1560, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.84 (3H, d,  $J=6\text{Hz}$ ), 1.34 (9H, s), 2.77 (s) and 2.87 (s) (3H), 2.5-3.2 (4H, m), 3.75-3.9 (2H, m), 4.18-5.20 (5H, m), 6.25 (1H, d,  $J=7\text{Hz}$ ), 6.9-7.7 (14H, m), 7.8-8.2 (2H, m), 8.4-8.8 (1H, m), 9.0-9.5 (1H, br s)

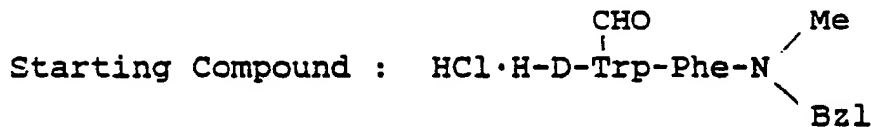
Elemental Analysis.

	Calculated for $\text{C}_{38}\text{H}_{45}\text{N}_5\text{O}_7$ :		
Found :	C 66.75, C 66.72,	H 6.63, H 6.55,	N 10.24 N 10.19

$[\alpha]_D^{25} + 39.03^\circ$  (c 1.135,  $\text{CHCl}_3$ )

### Example 14

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 13.



Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Boc-} \begin{array}{c} \text{NMe}_2 \\ | \\ \text{Glu} \end{array} \text{-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

mp : 95-100 °C

IR (Nujol) : 3280, 1750, 1720 (sh), 1710, 1690 (sh), 1655, 1640, 1560  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 1.4-2.1 (4H, m), 2.6-3.3 (4H, m), 2.67 (3H, s), 2.75 (3H, s), 3.8-4.2 (1H, m), 4.4-5.0 (2H, m), 5.14 (2H, s), 6.75 (1H, br s), 7.2-7.8 (4H, m), 7.25 (5H, s), 7.37 (5H, s), 7.8-8.4 (2H, m), 8.73 (1H, br d,  $J=8\text{Hz}$ ), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{40}\text{H}_{47}\text{N}_5\text{O}_8$ :		
Found :	C 66.19, C 66.38,	H 6.53, H 6.59,	N 9.65 N 9.21

(4)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Boc-Thr-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

mp : 158-160 °C

IR (Nujol) : 3340, 3290 (sh), 1720, 1685, 1640, 1540 (sh), 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.83 (3H, d,  $J=7\text{Hz}$ ), 1.33 (9H, s), 2.7-3.2 (4H, m), 3.7-4.1 (2H, m), 4.4-5.0 (3H, m), 5.10 (2H, s), 6.2-6.5 (1H, m), 7.2-7.8 (4H, m), 7.21 (5H, s), 7.33 (5H, s), 7.9-8.4 (2H, m), 8.62 (1H, br d,  $J=9\text{Hz}$ ), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{37}\text{H}_{42}\text{N}_4\text{O}_8$ :		
Found :	C 66.25, C 66.11,	H 6.31, H 6.20,	N 8.35 N 8.35

(5)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{Z-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

mp : 266-267 °C

IR (Nujol) : 3450, 3340, 3290, 1720, 1690, 1655, 1640, 1555, 1545 (sh)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.4-2.1 (4H, m), 2.6-3.2 (4H, m), 3.8-4.3 (1H, m), 4.4-4.9 (2H, m), 5.00 (2H, s), 5.12 (2H, s), 6.72 (1H, br s), 7.0-7.8 (6H, m), 7.23 (5H, s), 7.34 (10H, s), 8.10 (2H, br d,  $J=8\text{Hz}$ ), 8.69 (1H, br d,  $J=9\text{Hz}$ ), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{41}\text{H}_{41}\text{N}_5\text{O}_8$ :		
Found :	C 67.29, C 67.63,	H 5.65, H 5.42,	N 8.57 N 9.48

(6)

Starting Compound :  $\text{HCl} \cdot \text{H-D-Trp-Phe-OBzl}$

Object Compound :  $\text{Boc-Gln-D-Trp-Phe-OBzl}$

mp : 195-197 °C

4.8 (2H, m), 5.10 (2H, s), 6.70 (2H, br s), 7.20 (5H, s), 7.35 (5H, s), 7.1-7.7 (4H, m), 7.55 (1H, m), 7.95-8.25 (2H, m), 8.65 (1H, d, J=6Hz), 9.3 (1H, br s)  
Elemental Analysis.

Calculated for  $C_{38}H_{43}N_5O_8$  :

C 65.41, H 6.21, N 10.04

Found : C 65.14, H 6.09, N 9.96

$[\alpha]_D^{25} + 2.88^\circ$  (c 1.110, DMF)

### Example 12

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 11.

(1)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

Object Compound :  $Boc-Ser-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

mp : 164-166°C

IR (Nujol) : 3200, 1700 (broad), 1640, 1550, 1525  $cm^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 2.7-3.2 (4H, m), 3.35-3.65 (2H, m), 3.8-4.2 (1H, m), 4.4-4.9 (3H, m), 5.12 (2H, s), 6.60 (1H, br s), 7.2-7.7 (4H, m), 7.23 (5H, s), 7.36 (5H, s), 7.9-8.3 (2H, m), 8.67 (1H, br d, J=8Hz), 9.3 (1H, br s)

Elemental Analysis.

	Calculated for $C_{36}H_{40}N_4O_8 \cdot H_2O$		
	:		
Found :	C 64.08,	H 6.27,	N 8.30
	C 64.42,	H 6.28,	N 8.68

(2)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

Object Compound :  $Boc-Asn-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

mp : 208-210°C

IR (Nujol) : 3330, 1710, 1690, 1660, 1640, 1555 (sh), 1540  $cm^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 2.30 (2H, br d, J=6Hz), 2.6-3.2 (4H, m), 4.0-4.9 (3H, m), 5.12 (2H, s), 6.89 (2H, br s), 7.1-7.7 (5H, m), 7.24 (5H, s), 7.36 (5H, s), 7.93 (1H, br d, J=8Hz), 8.2 (1H, br s), 8.68 (1H, br d, J=8Hz), 9.3 (1H, br s) Elemental Analysis.

	Calculated for $C_{37}H_{41}N_5O_8$ :		
Found :	C 64.99,	H 6.04,	N 10.21
	C 65.36,	H 6.36,	N 10.21

(3)



	Calculated for $C_{35}H_{40}N_4O_5$ :		
Found :	C 70.45, C 70.49,	H 6.76, H 7.01,	N 9.39 N 9.18

Example 9

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 8.

Starting Compound : Boc-Phe-OCH<sub>2</sub>Py(2)

Object Compound : Boc-D-  $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$  -Phe-OCH<sub>2</sub>Py(2)

mp : 153-154 °C

IR (Nujol) : 3330, 1740, 1720, 1685, 1650, 1555, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.29 (9H, s), 2.55-2.85 (2H, m), 2.85-3.2 (2H, m), 4.1-4.5 (1H, m), 4.5-4.8 (1H, m), 5.22 (2H, s), 6.88 (1H, br d, J=9Hz), 7.2-7.6 (10H, m), 7.6-7.9 (2H, m), 7.9-8.3 (1H, m), 8.5-8.7 (2H, m), 9.4 (1H, broad)

Elemental Analysis.

	Calculated for $C_{32}H_{34}N_4O_6$ :		
Found :	C 67.35, C 67.38,	H 6.01, H 5.78,	N 9.82 N 9.82

Example 10

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 2.

Starting Compound : Boc-D-  $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$  -Phe-OCH<sub>2</sub>Py(2)

Object Compound : 2HCl•H-D-  $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$  -Phe-OCH<sub>2</sub>Py(2)

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.7-3.3 (4H, m), 3.9-4.5 (1H, m), 4.5-5.0 (1H, m), 5.44 (2H, s), 7.1-7.5 (7H, m), 7.5-7.9 (6H, m), 8.0-8.6 (4H, m), 8.6-8.9 (1H, m), 9.4 (1H, broad), 9.74 (1H, d, J=8Hz)

Example 11

Starting Compound : HCl•H-D-  $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$  -Phe-OBzl

Object Compound : Boc-Gln-D-  $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$  -Phe-OBzl

To a solution of Boc-Gln-OH (2.10 g), HCl•H-D-Trp(CHO)-Phe-OBzl (4.70 g) and HOBT (1.15 g) in a mixed solvent of methylene chloride (60 ml) and DMF (10 ml), was added WSC (1.41 g) under ice cooling.

The reaction mixture was stirred for 1.5 hours at the same temperature and for additional 1.5 hours at room temperature and concentrated under reduced pressure. Water was added to the residue and the resulting precipitates were collected and washed successively with water, diluted sodium hydrogencarbonate solution and water. After drying, the crude product (5.84 g) was stirred in hot ethyl acetate (60 ml) in water bath. After cooling, the precipitates were collected by filtration and dried to give Boc-Gln-D-Trp(CHO)-Phe-OBzl (5.70 g).

mp : 202-203.5 °C

IR (Nujol) : 3440, 3300, 1720, 1660 (sh), 1645  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 1.5-1.8 (2H, m), 1.85-1.95 (2H, m), 2.7-3.1 (4H, m), 3.90 (1H, br s), 4.45-

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 3.

Starting Compound :  $\text{Boc-D-}\overset{\text{Tos}}{\underset{|}{\text{Trp}}}\text{-OH}$

Object Compound :  $\text{Boc-D-}\overset{\text{Tos}}{\underset{|}{\text{Trp}}}\text{-Phe-N}\overset{\text{Me}}{\underset{\text{Bzl}}{|}}\text{}$

IR (Nujol) : 3300, 3250, 1710, 1620  $\text{cm}^{-1}$

mp : 98-100 °C

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 1.35 (9H, s), 2.28 (3H, s), 2.58 and 2.79 (3H, s), 2.74 (2H, d,  $J=6\text{Hz}$ ), 3.11 (2H, d,  $J=6\text{Hz}$ ), 4.22 and 4.60 (2H, ABq,  $J=14\text{Hz}$ ), 4.2-4.5 (1H, m), 4.85-5.2 (2H, m), 6.75-8.0 (20H, m)

Elemental Analysis.

	Calculated for $\text{C}_{40}\text{H}_{44}\text{N}_4\text{O}_6\text{S}_1$ :		
	C 67.78,	H 6.26,	N 7.90
Found :	C 67.24,	H 6.33,	N 7.62

#### Example 8

Starting Compound :  $\text{Boc-Phe-N}\overset{\text{Et}}{\underset{\text{Bzl}}{|}}\text{}$

Object Compound :  $\text{Boc-D-Trp-Phe-N}\overset{\text{CHO}}{\underset{|}{\text{Trp}}}\text{-N}\overset{\text{Et}}{\underset{\text{Bzl}}{|}}\text{}$

To an ice-cooled solution of Boc-Phe-NEtBzl (3.95 g) and anisole (4 ml) in methylene chloride (16 ml) was added TFA (16 ml). The solution was stirred for an hour at room temperature. After evaporation, addition and re-evaporation of 4N-HCl/DOX (5 ml) were repeated twice. The residue was dissolved in DMF (40 ml), and the solution was ice-cooled and neutralized with triethylamine (1.39 ml). To the solution containing H-Phe-NEtBzl obtained was added Boc-D-Trp(CHO)-OH (3.32 g), HOBT (1.35 g) and WSC $\cdot$ HCl (1.92 g). The solution was stirred for one and half an hour at room temperature. After evaporation and extraction with ethyl acetate. The organic layer was washed successively with water, 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride and dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (200 g) and eluted with a mixture of chloroform and methanol (50:1 to 20:1, gradient elution). The fractions containing the object compound were combined and evaporated. The residue were pulverized with n-hexane, collected by filtration, washed with n-hexane and dried to give Boc-D-Trp(CHO)-Phe-NEtBzl (4.47 g).

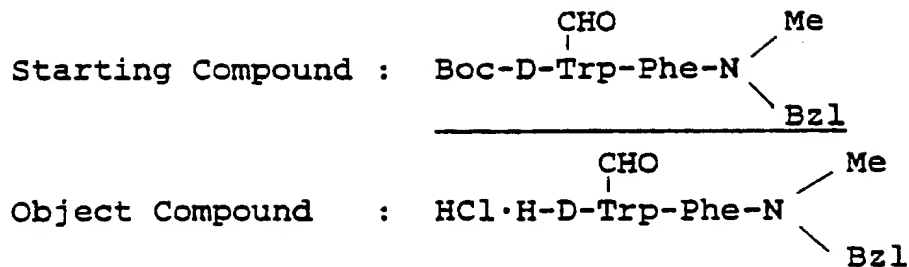
IR (Nujol) : 3300, 1710, 1630  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.97 (t,  $J=7\text{Hz}$ ) and 1.07 (t,  $J=7\text{Hz}$ )(3H), 1.25 (9H, s), 2.5-3.4 (6H, m), 4.1-5.2 (4H, m), 6.6-6.9 (1H, m), 6.9-7.9 (14H, m), 7.9-8.3 (1H, m) 8.56 (1H, br d,  $J=9\text{Hz}$ ), 9.3 (1H, broad)

Elemental Analysis.

$[\alpha]_D^{25} + 16.75^\circ$  (c 0.794  $\text{CHCl}_3$ )

#### Example 4



A mixture of Boc-D-Trp(CHO)-Phe-NMeBzl (1.53 g) and anisole (1.6 ml) was treated with TFA (10 ml) for 15 minutes under ice-cooling and for additional half an hour at room temperature. After evaporation of TFA, 4N-HCl/DOX (1.3 ml) was added to the residue and the mixture was concentrated again. The residue was triturated with ether, filtered, washed with diisopropyl ether, and dried to give HCl·H-D-Trp(CHO)-Phe-NMeBzl (13.4 g).

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 2.5-3.1 (4H, m), 2.81 (s) and 2.89 (s)(3H), 3.8-5.2 (4H, m), 6.9-7.5 (12H, m), 7.5-7.9 (2H, m), 8.2 (1H, br s), 8.4 (3H, br s), 9.1-9.6 (2H, m)

#### Example 5

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 1.

Starting Compound: Boc-D-Trp-Phe-OH

Object Compound : Boc-D-Trp-Phe-OBzl

mp : 145-146°C

IR (Nujol) : 145-146°C

IR (Nujol) : 3400 (sh), 3360, 1730, 1690, 1660, 1520  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 1.30 (9H, s), 2.5-3.3 (4H, m), 4.00-4.35 (1H, m), 4.35-4.75 (1H, m), 5.08 (2H, s), 6.55 (1H, d,  $J=8.5\text{Hz}$ ), 6.80-7.65 (16H, m), 8.36 (1H, d,  $J=8.5\text{Hz}$ )

Elemental Analysis.

	Calculated for $\text{C}_{32}\text{H}_{35}\text{N}_3\text{O}_5$ :		
	C 70.96,	H 6.51,	N 7.76
Found :	C 71.12,	H 6.76,	N 7.88

#### Example 6

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 2.

Starting Compound : Boc-D-Trp-Phe-OBzl

Object Compound : HCl·H-D-Trp-Phe-OBzl

IR (Nujol) : 3400 (broad), 3200, 1735, 1690 (sh), 1680  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 2.55-3.25 (4H, m), 3.75-4.15 (1H, m), 4.30-4.60 (1H, m), 5.03 (2H, s), 6.6-7.70 (15H, m), 8.07 (3H, br s), 9.13 (1H, d,  $J=9\text{Hz}$ ), 10.93 (1H, s)

#### Example 7

and 9Hz), 4.2-4.5 (1H, m), 4.5-4.85 (1H, m), 5.15 (2H, s), 6.83 (1H, d, J=8Hz), 7.25 (5H, s), 7.40 (5H, s), 7.2-7.85 (4H, m), 8.20 (1H, br s), 8.62 (1H, d, J=8Hz), 9.3-9.8 (1H, br s)  
Elemental Analysis.

5

	Calculated for $C_{33}H_{35}N_3O_6$ :		
	C 69.58,	H 6.19,	N 7.38
Found :	C 69.69,	H 6.09,	N 7.36

10

Example 2

15

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

20

TFA (45 ml) was added to a mixture of Boc-D-Trp(CHO)-Phe-OBzl (4.86 g) and anisole (6.0 ml) under ice cooling and the mixture was stirred for 15 minutes at the same temperature and for additional 20 minutes after removing the ice bath. The reaction mixture was concentrated and 4N-HCl/DOX (4.27 ml) was added, and concentrated again. Addition of diisopropyl ether gave precipitates, which were collected by filtration, washed with the same solvent, and dried to give HCl·H-D-Trp(CHO)-Phe-OBzl (4.70 g).  
NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.7-3.3 (4H, m), 3.9-4.3 (1H, m), 4.4-4.9 (1H, m), 5.13 (2H, s), 7.23 (5H, s), 7.36 (5H, s), 7.2-7.5 (2H, m), 7.55-7.85 (2H, m), 8.2 (1H, br s), 8.35 (3H, br s), 9.4 (1H, br s), 9.45 (1H, br d, J=8Hz)

25

Example 3

30

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

Object Compound :  $\text{Boc-D-Trp-Phe-N} \begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$

35

Boc-D-Trp(CHO)-OH (3.26 g), HCl·H-Phe-NMeBzl (2.99 g) and HOBT (1.32 g) were dissolved in DMF (40 ml). To this solution was added WSC under ice cooling. The reaction mixture was stirred for an hour at this temperature and for additional an hour at room temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with diluted sodium hydrogencarbonate solution, water, 0.5N hydrochloric acid, and sodium chloride solution and dried over magnesium sulfate. The evaporated residue was crystallized from a mixed solvent of ethyl acetate and diisopropyl ether (3:4) (35 ml) with seeding. The crystals were collected by filtration after addition of diisopropyl ether (55 ml) and dried to give Boc-D-Trp(CHO)-Phe-NMeBzl (4.96 g).

mp : 88-90°C

IR (Nujol) : 3300-3200, 1710, 1620, 1530  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ )  $\delta$  : 1.41 (9H, s), 2.70 and 2.85 (3H, s), 2.90 (2H, d, J=7Hz), 3.18 (2H, d, J=7Hz), 4.2-4.73 (3H, m), 4.98-5.28 (2H, m), 6.9-7.4 (14H, m), 7.5-7.7 (1H, m), 8.3 (1H, br s), 8.8-9.5 (1H, br s)

Elemental Analysis.

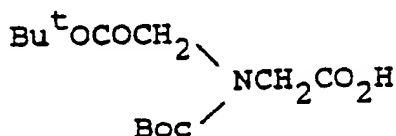
50

Calculated for  $C_{34}H_{38}N_4O_5$   
C 70.08, H 6.57, N 9.62

Found : C 70.39, H 6.86, N 9.49

55

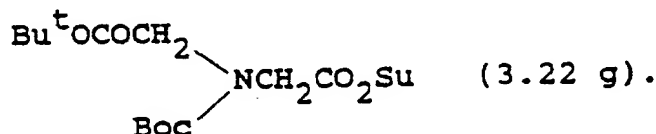
$[\alpha]_D^{25} +$



5

(3.95 g) and pyridine (1.08 g) in acetonitrile (50 ml) was added di-succinimidyl carbonate (3.49 g). The solution was stirred overnight at room temperature. After concentration, the product was extracted with ethyl acetate and the extract was washed successively with water, diluted sodium hydrogencarbonate solution, 0.5N hydrochloric acid, and sodium chloride solution, and dried over magnesium sulfate. The residue (3.84 g) was crystallized with diisopropyl ether-n-hexane (1:1) to give

15



20

mp : 102-108° C

IR (Nujol) : 1840, 1780, 1745 (sh), 1730 cm<sup>-1</sup>

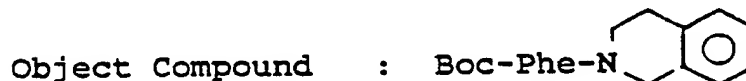
NMR (CDCl<sub>3</sub>, δ) : 1.50 (18H, s), 2.87 (4H, s), 4.02 and 4.38 (4H, two set of ABq, J = 10Hz)

25

#### Preparation 22

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Preparation 1-(1).

30 Starting Compound : Boc-Phe-OH



35

NMR (DMSO-d<sub>6</sub>, δ) : 1.29 (s) and 1.30 (s) (9H), 2.5-3.0 (4H, m), 3.4-3.8 (2H, m), 4.4-4.8 (3H, m), 6.7-6.9 (1H, m), 7.0-7.3 (9H, m)

40

#### Example 1

Starting Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

45

Object Compound : Boc-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

50

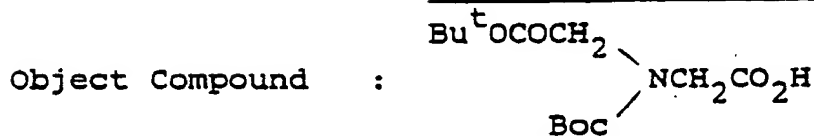
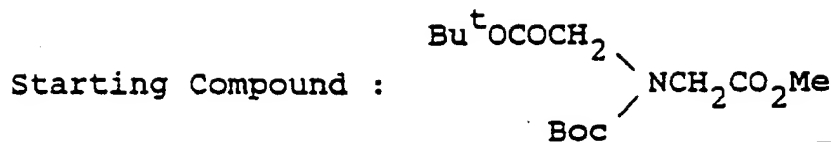
Boc-D-Trp(CHO)-OH (2.99 g), TsOH·H-Phe-OBzl (3.85 g) and HOBT (1.22 g) were dissolved in a mixed solvent of methylene chloride (60 ml) and DMF (15 ml). To this solution was added WSC (1.53 g) under ice cooling, and the reaction mixture was stirred for 3 hours at the same temperature. The reaction mixture was concentrated and extracted with ethyl acetate. The organic layer was washed successively with diluted sodium hydrogencarbonate solution (twice), water, 0.5N hydrochloric acid, and saturated sodium chloride solution, and dried over magnesium sulfate. After concentration, the residue was crystallized from a mixture of ethyl acetate and diisopropyl ether (1:1), which was filtered, washed with diisopropyl ether, and dried to give Boc-D-Trp(CHO)-Phe-OBzl (4.95 g).

55

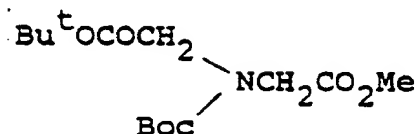
mp : 146-147° C

IR (Nujol) : 3340, 1732 (sh), 1710, 1686, 1650, 1545, 1528 cm<sup>-1</sup>

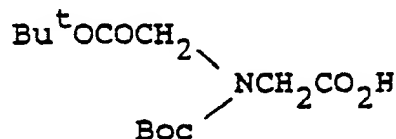
NMR (DMSO-d<sub>6</sub>, δ) : 1.30 (9H, s), 2.65-2.85 (2H, m), 2.90 and 3.15 (2H, d of ABq, J = 14Hz and 6Hz, 14Hz



To an ice-cooled solution of



(3.9 g) in methanol (40 ml) was added dropwise 1N-sodium hydroxide solution (10 ml). After stirring for two hours 1N-sodium hydroxide solution (7 ml) was added. After evaporation of methanol, water (20 ml) was added and extracted with ether once. The aqueous layer was acidified to pH 2, and extracted with ethyl acetate and the organic layer was washed with sodium chloride solution and dried over magnesium sulfate to give

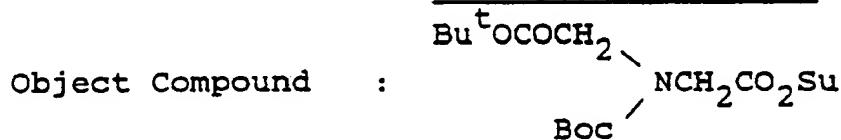
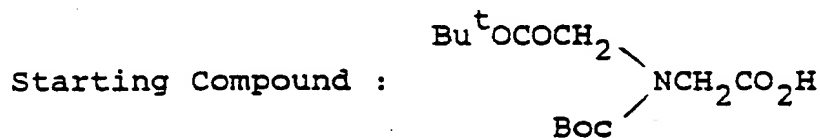


(3.02 g) as an oil.

IR (Film) : 2600, 1740-1700 (br)  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 1.43 (9H, s), 1.50 (9H, s), 3.95-4.3 (4H, m), 9.43, (1H, s)

#### Preparation 21



To an ice-cooled solution of

Preparation 18

Starting compound :  $\text{Boc}-\overset{\text{OTce}}{\underset{\text{Glu}}{\text{Glu}}}-\text{OBzl}$

5 Object Compound :  $\text{Boc}-\overset{\text{OTce}}{\underset{\text{Glu}}{\text{Glu}}}-\text{OH}$

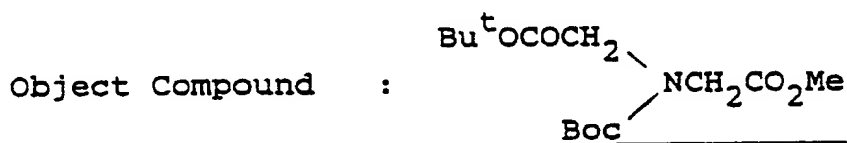
Boc-Glu(OTce)-OBzl (0.50 g) was hydrogenated in ethanol (25 ml) with 10 % palladium on carbon (0.10 g). The catalyst was filtered off and the filtrate was evaporated. The residue was extracted with ethyl acetate. The organic layer was washed successively with 2% hydrochloric acid, water and saturated sodium chloride, dried over magnesium sulfate and evaporated. The residue was pulverized with petroleum ether, filtered and dried to give Boc-Glu(OTce)-OH (0.30 g).

IR (Nujol) : 3400, 1740, 1730, 1660, 1520  $\text{cm}^{-1}$

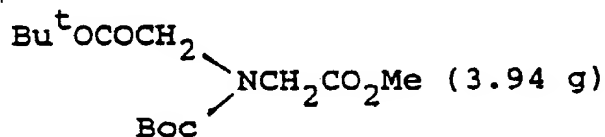
NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.38 (9H, s), 1.7-2.2 (2H, m), 2.3-2.6 (2H, m), 3.8-4.2 (1H, m), 4.88 (2H, s), 7.12 (1H, br d,  $J=8\text{Hz}$ ), 12.5 (1H, broad)

Preparation 19

Starting Compound : Boc-Gly-OMe



To an ice-cooled solution of Boc-Gly-OMe (1.89 g) and tert-butyl bromoacetate (3.90 g) in THF (30 ml) was added sodium hydride (60% in oil) (0.8 g) under nitrogen atmosphere. The solution was stirred for an hour under ice-cooling and further for two hours at room temperature. Acetic acid (1.5 ml) was added to the solution under cooling and the produce was extracted with ethyl acetate. The organic layer was washed successively with 0.5N hydrochloric acid, diluted sodium hydrogencarbonate solution, and sodium chloride solution, and dried over magnesium sulfate to give

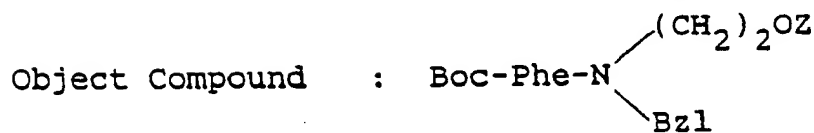
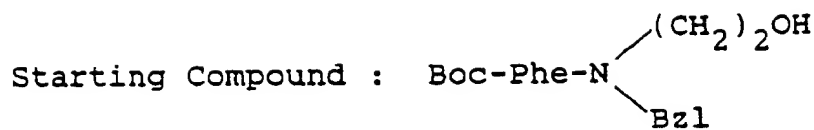


as an oil.

IR (film) : 1750, 1710  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) 1.15 (9H x 2, s), 3.77 (3H, s), 3.97 (2H, dd,  $J=15\text{Hz}$ ), 4.08 (2H, dd,  $J=15\text{Hz}$ )

Preparation 20



To a solution of Boc-Phe-N((CH<sub>2</sub>)<sub>2</sub>OH)Bzl (3.75 g), pyridine (7.6 ml) and 4-dimethylaminopyridine (0.23 g) in THF (100 ml) was added dropwise a solution of benzyl chloroformate (2.7 ml) in THF (3 ml) under ice-cooling. After stirring for 2 hours, a solution of benzyl chloroformate (2.7 ml) in THF (3 ml) was added to the mixture. The mixture was stirred for further 3 hours and then evaporated. The residue was crystallized with petroleum ether, filtered, washed with petroleum ether and dried to give Boc-Phe-N((CH<sub>2</sub>)<sub>2</sub>OZ)Bzl (4.58 g).  
mp : 85-86 °C

IR (Nujol) : 3390, 1740, 1690, 1650, 1520 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.25 (s) and 1.32 (s)(9H), 2.6-3.0 (2H, m), 3.2-3.8 (2H, m), 3.8-4.9 (5H, m), 5.10 (2H, s), 6.9-7.5 (16H, m)

#### Preparation 16

Starting Compound : Boc-Phe-OPy(2)

Object Compound : Boc-Phe-(CH<sub>2</sub>)<sub>2</sub>Ph

In a nitrogen atmosphere, a solution of phenethyl bromide (2.05 ml) in THF (10 ml) was added to a stirred mixture of magnesium (0.44 g) in THF (5 ml) at 30-40 °C. After filtration, the solution was added over fifteen minutes to a stirred solution of Boc-Phe-OPy(2) (1.71 g) in THF (100 ml) at -70 °C. The mixture was stirred for half an hour at -70 °C, then saturated ammonium chloride solution (15 ml) was added. After filtration, evaporation and extraction with ethyl acetate, the organic layer was washed with 0.1N sodium hydroxide solution and saturated sodium chloride solution, dried over magnesium sulfate and evaporated. The residual solid was filtered, washed with n-hexane. The solid was subjected to column chromatography on silica gel (200 g) and eluted with a mixture of chloroform and n-hexane (1:1). The fractions containing the object compound were combined and evaporated. The residual white crystals were filtered washed with n-hexane and dried to give Boc-Phe-(CH<sub>2</sub>)<sub>2</sub>Ph (1.30 g).

IR(Nujol) : 3460, 1715, 1960, 1515 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.31 (9H, s), 2.6-3.2 (2H, m), 2.76 (4H, s), 4.0-4.4 (1H, m), 7.22 (11H, s)

#### Preparation 17

Starting Compound : Boc-Glu-OBzl

Object compound : Boc-  $\begin{array}{c} \text{OTce} \\ | \\ \text{Glu} \end{array}$  - OBzl

To a solution of Boc-Glu-OBzl (1.00 g) and TceOH (0.53 g) in methylene chloride (15 ml) were added 4-dimethylaminopyridine (0.04 g) and WSC·HCl (0.57 g) successively under ice cooling. The mixture was stirred for 3 hours at the same temperature. After evaporation, the residue was extracted with ethyl acetate. The organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate solution, water and saturated sodium chloride solution, and dried over magnesium sulfate. The evaporated residue was crystallized with petroleum ether, filtered and dried to give Boc-Glu(OTce)-OBzl (1.01 g).

IR (Nujol) : 3400, 1740, 1700, 1510 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) 1.36 (9H, s), 1.7-2.2 (2H, m), 2.4-2.7 (2H, m), 3.9-4.3 (1H, m), 4.88 (2H, s), 5.14 (2H, s), 7.3 (1H, br s), 7.38 (5H, s)



Starting Compound : Boc-Phe-OH

Object Compound : Boc-Phe-NHBzl

IR (Nujol) : 3310, 1680, 1660, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 2.6-3.2 (2H, m), 4.0-4.4 (1H, m), 4.30 (2H, d J=6Hz), 6.92 (1H, br d, J=8Hz), 7.28 (10H, s), 8.40 (1H, t, J=6Hz)

(2)

Starting Compound : Boc-Phe-OH

Object Compound : Boc-Phe-NHPh

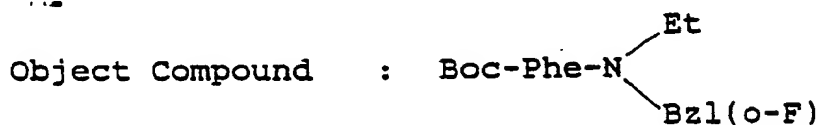
NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.32 (9H, s), 2.6-3.2 (2H, m), 4.0-4.5 (1H, m), 6.9-7.5 (9H, m), 7.5-7.7 (2H, m), 10.09 (1H, s)

#### Preparation 14

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Preparation 1-(1).

(1)

Starting Compound : Boc-Phe-OH

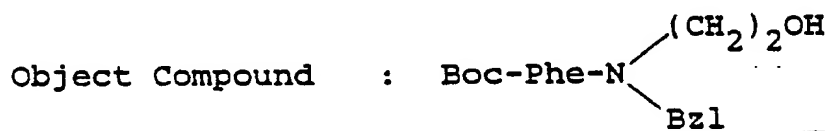


IR (Neat) : 1710, 1640, 1490  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.89 (t, J=6.5Hz) and 0.97 (t, J=6.5Hz) (3H), 1.25 (s) and 1.33 (s) (9H), 2.7-3.1 (2H, m), 3.28 (q, J=6.5Hz), and 3.43 (q, J=6.5Hz) (2H), 4.3-4.8 (3H, m), 6.9-7.4 (5H, m), 7.20 (5H, s)

(2)

Starting Compound : Boc-Phe-OH



IR (Nujol) : 3460, 3390, 1960, 1625, 1520  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.25 (s) and 1.32 (s) (9H), 2.6-3.8 (6H, m), 4.2-4.9 (4H, m), 6.9-7.4 (11H, m)

#### Preparation 15

Starting Compound : Boc-Phe-NHPh

Object Compound : HCl•H-Phe-NHPh

NMR (DMSO- $d_6$ ,  $\delta$ ) : 3.88 (2H, d,  $J$  = Hz), 4.36 (1H, t,  $J$  = 6Hz), 7.0-7.5 (8H, m), 7.5-7.7 (2H, m), 8.52 (3H, br s), 11.00 (1H, s)

5

(5)

Starting Compound : Boc-Phe-(CH<sub>2</sub>)<sub>2</sub>Ph

Object Compound : HCl•H-Phe-(CH<sub>2</sub>)<sub>2</sub>Ph

10 IR (Nujol) : 3200, 1720, 1610 cm<sup>-1</sup>

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.6-2.9 (4H, m), 3.0-3.3 (2H, m), 4.37 (1H, t,  $J$  = 7Hz), 7.0-7.4 (5H, m), 7.30 (5H, s), 8.61 (3H, br s)

#### Preparation 11

15

Starting Compound : Boc-Phe-OH

Object Compound : Boc-Phe-OCH<sub>2</sub>Py(3)

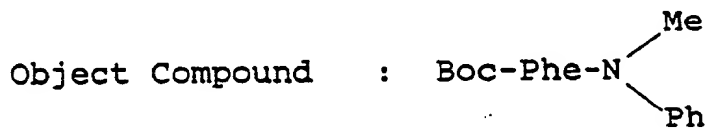
20 To a solution of Boc-Phe-OH (2.65 g) and 3-pyridinemethanol (1.31 g) in DMF (30 ml) were added WSC•HCl (1.92 g) and 4-dimethylaminopyridine (0.12 g) under ice-cooling. The mixture was stirred for 3.5 hours. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with water, 2% sodium hydrogencarbonate, water and saturated sodium chloride solution, dried over magnesium sulfate and evaporated. The residue was subjected to column chromatography on silica gel (50 g), and eluted with chloroform and then a mixture of chloroform and methanol (50:1). The fractions containing the

25 object compound were combined and evaporated to give Boc-Phe-OCH<sub>2</sub>Py(3) (3.56 g).  
NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 2.7-3.1 (2H, m), 3.9-4.4 (1H, m), 5.15 (2H, s), 7.1-7.5 (2H, m), 7.28 (5H, s), 7.6-7.8 (1H, m), 8.5-8.7 (2H, m)

#### 30 Preparation 12

Starting Compound : Boc-Phe-OH

35



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To a solution of Boc-Phe-OH (2.65 g), N-methylaniline (1.09 g) and HOBT (1.35 g) in DMF (25 ml) was added WSC•HCl (1.92 g) under ice-cooling. The mixture was stirred for 5 hours at room temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride solution, and dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (100 g) and eluted with a mixture of chloroform and methanol (100:1). The fractions containing the

45

object compound were combined and evaporated to give Boc-Phe-NMePh (2.17 g).

IR (Neat) : 3310, 2290, 1710, 1655, 1600, 1500 cm<sup>-1</sup>

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 2.5-3.0 (2H, m), 3.17 (3H, s), 4.0-4.4 (1H, m), 6.6-7.6 (11H, m)

50

#### Preparation 13

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Preparation 12.

55

(1)

similar manner to that of Preparation 7.

Starting Compound : H-Phe-OH

Object Compound : TsOH·H-Phe-OCH<sub>2</sub>cHex

IR (Nujol) : 1735, 1515, 1240, 1210, 1180 cm<sup>-1</sup>

- 5 NMR (DMSO-d<sub>6</sub>, δ) : 0.51-1.7 (11H, m), 2.30 (3H, s), 2.8-3.5 (2H, m), 3.86 (2H, d, J=6Hz), 4.33 (1H, dd, J=6 and 8Hz), 7.15 (2H, d, J-8Hz), 7.2-7.5 (5H, m), 7.55 (2H, d, J-8Hz), 8.48 (3H, br s)

#### Preparation 9

10

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Preparation 4.

Starting Compound : Boc-Phe-OH

Object Compound : Boc-Phe-OCH<sub>2</sub>Py(4)

- 15 IR (Nujol) : 3210, 1750, 1705, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.33, (9H, s), 2.8-3.2 (2H, m), 4.1-4.5 (1H, m), 5.16 (2H, s), 7.1-7.5 (3H, m), 7.28 (5H, s) 8.5-8.6 (2H, m)

#### 20 Preparation 10

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Preparation 1-(2).

25

(1)

Starting Compound: Boc-Phe-OCH<sub>2</sub>Py(4)

Object Compound : 2HCl·H-Phe-OCH<sub>2</sub>Py(4)

- 30 NMR (DMSO-d<sub>6</sub>, δ) : 3.0-3.6 (2H, m), 4.3-4.6 (1H, m), 5.46 (2H, s), 7.33 (5H, s), 7.92 (2H, d, J-6Hz), 8.92 (2H, d, J-6Hz), 9.2 (4H, br s)

(2)

35 Starting Compound : Boc-Phe-NHBzl

Object Compound : HCl·H-Phe-NHBzl

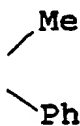
IR (Nujol) : 3430, 1670, 1545 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 3.13 (2H, d, J=6Hz), 4.0-4.5 (3H, m), 7.0-7.4 (5H, m), 7.28 (5H, s), 8.58 (3H, br s), 9.19 (1H, br t, J=6Hz)

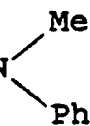
40

(3)

45

Starting Compound : Boc-Phe-N 

50

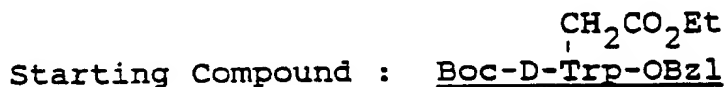
Object Compound : HCl·H-Phe-N 

- 55 NMR (DMSO-d<sub>6</sub>, δ) : 2.91 (2H, d, J=6Hz), 3.10 (3H, s), 3.79 (1H, t, J=6Hz), 6.6-7.0 (4H, m), 7.1-7.4 (6H, m), 8.67 (3H, s)

(4)

Preparation 5Starting Compound : Boc-D-Trp-OBzl

To solution of Boc-D-Trp-OBzl (3.0 g) in methylene chloride (60 ml) were added powdered sodium hydroxide (1.52 g), ethyltrimethylammonium chloride (150 mg) and ethyl bromoacetate 2.54 g). The mixture was stirred overnight at room temperature, then powdered sodium hydroxide (0.61 g) and ethyl bromoacetate (0.63 g) were added. The mixture was stirred further for four and half an hour at room temperature and for two hours under reflux. After cooling, 1N-hydrochloric acid (53 ml) was added to the mixture, and the organic layer was washed with sodium chloride solution and dried with magnesium sulfate. After evaporation, the residue (4.87 g) was chromatographed on a silica gel column (60 g) eluting successively with chloroform and chloroform-ethyl acetate (4:1) to give a purified Boc-D-Trp(CH<sub>2</sub>CO<sub>2</sub>Et)-OBzl (4.14 g).  
NMR (CDCl<sub>3</sub>, δ) : 1.20 (3H, t, J = 7Hz), 1.43 (9H, s), 3.31 (2H, d, J = 6Hz), 4.22 (2H, q, J = 7Hz), 4.70 (2H, s), 5.11 (2H, s), 4.7 (1H, m), 5.1 (1H, m), 6.7 (1H, s), 7.1-7.4 (3H, m), 7.3 (5H, s), 7.5-7.7 (1H, m)

Preparation 6

To a solution of Boc-D-Trp(CH<sub>2</sub>CO<sub>2</sub>Et)-OBzl (4.14 g) in ethanol (60 ml) was added 5% palladium on carbon (0.7 g) and the mixture was hydrogenated for one and half an hour under atmospheric pressure. Filtration of the catalyst and concentration of the filtrate under vacuum gave Boc-D-Trp(CH<sub>2</sub>CO<sub>2</sub>Et)-OH as an amorphous solid (3.06 g).  
NMR (CDCl<sub>3</sub>, δ) : 1.23 (3H, t, J = 7Hz), 1.40 (9H, s), 3.32 (2H, d, J = 6Hz), 4.23 (2H, q, J = 7Hz), 4.77 (2H, s), 4.6-4.8 (1H, m), 5.2 (1H, m), 7.00 (1H, s), 7.1-7.4 (2H, m), 7.6-7.9 (2H, m)

Preparation 7Starting Compound : H = Phe-OHObject Compound : TsOH·H-Phe-OBzl(Cl)

A mixture of H-Phe-OH (1.65 g), 4-chlorobenzyl alcohol (7.12 g) and p-toluenesulfonic acid monohydrate (2.09 g) in carbon tetrachloride (30 ml) was refluxed for 22 hours while water was removed by molecular sieves 3A1/8. After adding diethyl ether, the white crystal was filtered, washed with diethyl ether and dried to give TsOH·H-Phe-OBzl(Cl) (4.59 g).  
IR (Nujol) : 3250, 1750, 1600, 1520 cm<sup>-1</sup>  
NMR (DMSO-d<sub>6</sub>, δ) : 2.29 (3H, s), 2.9-3.4 (2H, m), 4.37 (1H, t, J = Hz), 5.13 (2H, s), 7.1-7.7 (13H, m), 8.51 (3H, br, s)

Preparation 8

The following object compound was obtained from the corresponding starting compound according to a

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-O(CH}_2\text{)}_2\text{Ph}$

IR (Nujol) : 1710, 1690  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.6-3.2 (4H, m), 2.87 (2H, t,  $J = 7\text{Hz}$ ), 3.9-4.7 (2H, m), 4.27 (2H, t,  $J = 7\text{Hz}$ ), 7.1-7.5 (2H, m), 7.19 (5H, s), 7.30 (5H, s), 7.6-7.9 (2H, m), 8.0-8.4 (1H, m), 8.35 (3H, br s), 9.4 (1H, broad), 9.41 (1H, d,  $J = 7\text{Hz}$ )

(4)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl(Cl)}$

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl(Cl)}$

IR (Nujol) : 1710, 1690, 1600  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.7-3.4 (4H, m), 4.0-4.3 (1H, m), 4.4-4.8 (1H, m), 5.14 (2H, s), 7.2-7.6 (6H, m), 7.26 (5H, s), 7.6-7.9 (2H, m), 8.2 (1H, broad), 8.42 (3H, br s), 9.4 (1H, broad), 9.54 (1H, br d,  $J = 8\text{Hz}$ )

(5)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{cHex}$

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{cHex}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.6-1.8 (10H, m), 2.6-3.3 (5H, m), 3.85 (2H, d,  $J = 6\text{Hz}$ ), 4.13 (1H, br t,  $J = 6\text{Hz}$ ), 4.57 (1H, br q,  $J = 7\text{Hz}$ ), 7.1-7.5 (2H, m), 7.25 (5H, s), 7.6-7.8 (2H, m), 8.2 (1H, br s), 8.4 (3H, br s), 9.4 (1H, broad), 9.43 (1H, d,  $J = 8\text{Hz}$ )

(6)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(4)}$

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(4)}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.7-3.4 (4H, m), 4.0-4.4 (1H, m), 4.5-4.9 (1H, m), 5.43 (2H, s), 7.1-7.5 (3H, m), 7.30 (5H, s), 7.5-7.9 (2H, m), 7.96 (2H, d,  $J = 6\text{Hz}$ ), 8.0-8.3 (1H, m), 8.5 (3H, br s), 8.92 (2H, d,  $J = 6\text{Hz}$ ), 9.45 (1H, broad), 9.82 (1H, br d,  $J = 8\text{Hz}$ )

(7)

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-(CH}_2\text{)}_2\text{Ph}$

Object Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-(CH}_2\text{)}_2\text{Ph}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.6-3.3 (8H, m), 3.9-4.3 (1H, m), 4.4-4.8 (1H, m), 7.0-7.5 (2H, m), 7.20 (10H, s), 7.5-7.8 (2H, m), 8.2 (1H, br s), 8.3 (3H, br s), 9.4 (1H, broad), 9.49 (1H, d,  $J = 8\text{Hz}$ )

#### Example 48

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 11.

(1)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OMe}$

Object Compound :  $\text{Boc-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OMe}$

mp : 165-167 °C

IR (Nujol) : 3310, 1710, 1690, 1650 (broad), 1540, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 1.4-2.1 (4H, m), 2.6-3.1 (4H, m), 3.63 (3H, s), 3.7-4.1 (1H, m), 4.3-4.8 (2H, m), 6.6-6.9 (2H, m), 7.0-7.5 (4H, m), 7.25 (5H, s), 7.5-7.7 (1H, m), 7.9-8.3 (2H, m), 8.64 (1H, br d,  $J = 8\text{Hz}$ ), 9.3 (1H, broad)

## Elemental Analysis.

	Calculated for $C_{32}H_{39}N_5O_8 \cdot 2/3H_2O$ :		
Found :	C 60.65, C 60.59,	H 6.41, H 6.06,	N 11.05 N 10.97

(2)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OPr^i$

Object Compound : Boc-Gln-D-Trp-Phe-OPr<sup>i</sup>

mp : 213-216 °C

IR (Nujol) : 3450, 3350, 1715, 1690, 1660, 1645, 1545, 1530  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.07 (3H, d, J=7Hz), 1.17 (3H, d, J=7Hz), 1.32 (9H, s), 1.5-2.2 (4H, m), 2.6-3.2 (4H, m), 3.8-4.1 (1H, m), 4.3-4.9 (2H, m), 4.88 (1H, sep, J=7Hz), 6.6-7.0 (2H, m), 7.0-7.6 (4H, m), 7.23 (5H, s), 7.6-7.8 (1H, m), 7.9-8.3 (2H, m), 8.70 (1H, br d, J=8Hz), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{34}H_{43}N_5O_8$ :		
Found :	C 62.85, C 63.11,	H 6.67, H 7.00,	N 10.78 N 10.54

(3)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-O(CH_2)_2Ph$

Object Compound : Boc-Gln-D- $\overset{\overset{CHO}{|}}{Trp}$ -Phe-O(CH<sub>2</sub>)<sub>2</sub>Ph

mp : 157-159 °C

IR (Nujol) : 3330, 1725, 1710, 1690, 1645, 1530  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 1.5-2.2 (4H, m), 2.6-3.1 (6H, m), 3.7-4.2 (1H, m), 4.27 (2H, t, J=6Hz), 4.4-4.9 (2H, m), 6.6-6.9 (2H, m), 7.0-7.8 (5H, m), 7.22 (5H, s), 7.28 (5H, s), 7.9-8.3 (2H, m), 8.61 (1H, br d, J=8Hz), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{39}H_{45}N_5O_8$ :		
Found :	C 65.81, C 65.76,	H 6.37, H 6.75,	N 9.84 N 9.73

(4)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl(Cl)$

Object Compound : Boc-Gln-D- $\overset{\overset{CHO}{|}}{Trp}$ -Phe-OBzl(Cl)

mp : 214-216 °C

IR (Nujol) : 3310, 1725, 1710, 1685, 1640, 1545, 1530  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.32 (9H, s), 1.4-2.2 (4H, m), 2.6-3.2 (4H, m), 3.8-4.1 (1H, m), 4.4-4.9 (2H, m), 5.11 (2H, s), 6.6-6.9 (2H, m), 7.0-7.7 (9H, m), 7.23 (5H, s), 7.9-8.4 (2H, m), 8.73 (1H, br d, J=9Hz), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{38}H_{42}ClN_5O_8$ :		
Found :	C 62.33, C 62.28,	H 5.78, H 5.75,	N 9.56 N 9.57

5

(5)

Starting Compound :  $HCl \cdot H-D-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OCH}_2\text{cHex}$

Object Compound :  $\text{Boc-Gln-D}-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OCH}_2\text{cHex}$   
 mp : 199-201 °C

IR (Nujol) : 3340, 1710, 1690, 1655, 1645, 1545, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.6-2.1 (14H, m), 1.33 (9H, s), 2.7-3.3 (5H, m), 3.7-4.1 (1H, m), 3.84 (2H, d, J=6Hz), 4.3-4.9 (2H, m), 6.6-6.9 (2H, m), 7.0-7.8 (5H, m), 7.25 (5H, s), 7.9-8.4 (2H, m), 8.5-8.8 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

20

	Calculated for $C_{38}H_{49}N_5O_8 \cdot 1/2H_2O$ :		
Found :	C 64.03, C 64.10,	H 7.07, H 6.96,	N 9.82 N 9.75

25

(6)

Starting Compound :  $2HCl \cdot H-D-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OCH}_2\text{Py(4)}$

Object Compound :  $\text{Boc-Gln-D}-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OCH}_2\text{Py(4)}$   
 mp : ~169 °C (dec.)

IR (Nujol) : 3330, 1710, 1690, 1660, 1640, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 1.4-2.2 (4H, m), 2.6-3.2 (4H, m), 3.7-4.1 (1H, m), 4.5-4.9 (2H, m), 5.16 (2H, s), 6.6-6.9 (2H, m), 7.0-7.7 (7H, m), 7.24 (5H, s), 7.9-8.3 (2H, m), 8.5-8.6 (2H, m), 8.72 (1H, br d, J=7Hz), 9.3 (1H, broad)

Elemental Analysis.

40

	Calculated for $C_{37}H_{42}N_5O_8 \cdot 1/2H_2O$ :		
Found :	C 62.79, C 62.88,	H 6.12, H 5.96,	N 11.87 N 11.87

45

(7)

Starting Compound :  $HCl \cdot H-D-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OBzl}$

Object Compound :  $\text{Boc-Gly-D}-\overset{\text{CHO}}{\underset{|}{\text{Trp}}}-\text{Phe-OBzl}$   
 mp : 78-80 °C

IR (Nujol) : 3290, 1750, 1710, 1650, 1555  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 2.6-3.3 (4H, m), 3.49 (2H, d, J=6Hz), 4.4-4.9 (2H, m), 5.13 (2H, s), 6.9 (1H, br s), 7.2-7.8 (4H, m), 7.24 (5H, s), 7.37 (5H, s), 7.97 (1H, d, J=9Hz), 8.2 (1H, broad), 8.76 (1H, d, J=9Hz), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{35}H_{38}N_4O_7$ :		
Found :	C 67.08, C 66.83,	H 6.11, H 5.58,	N 8.94 N 8.93

(8)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

Object Compound :  $Boc-Tyr-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

mp : 213-215 °C

IR (Nujol) : 3450, 3290, 1755, 1715, 1640, 1560  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.25 (9H, s), 2.3-2.6 (2H, m), 2.6-3.2 (4H, m), 3.9-4.3 (1H, m), 4.4-5.0 (2H, m), 5.12 (2H, s), 6.4-6.7 (1H, m), 6.53 (2H, d, J = 9Hz), 6.86 (2H, d, J = 9Hz), 7.2-7.8 (4H, m), 7.26 (5H, s), 7.35 (5H, s), 8.0-8.4 (2H, m), 8.6-8.9 (1H, m), 9.08 (1H, s), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{42}H_{44}N_4O_8$ :		
Found :	C 68.84, C 68.62,	H 6.05, H 6.09,	N 7.65 N 7.67

(9)

Starting Compound :  $HCl \cdot H-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

Object Compound :  $H_2NCO(CH_2)_2CO-D-\overset{\overset{CHO}{|}}{Trp}-Phe-OBzl$

mp : 199-200 °C

IR (Nujol) : 3430, 3300, 1735, 1715, 1665, 1645, 1535  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.24 (3H, s), 2.6-3.3 (4H, m), 4.5-4.8 (2H, m), 5.11 (2H, s), 6.74 (1H, br s), 7.1-7.8 (5H, m), 7.20 (5H, s), 7.35 (5H, s), 8.10 (2H, br d, J = 9Hz), 8.65 (1H, d, J = 8Hz), 9.35 (1H, broad)

Elemental Analysis.

	Calculated for $C_{32}H_{32}N_4O_6$ :		
Found :	C 67.59, C 67.45,	H 5.67, H 5.62,	N 9.85 N 9.96

(10)

Starting Compound :  $HCl \cdot H-D-Trp-Phe-OBzl$ Object Compound :  $Boc-D-Trp-D-Phe-OBzl$ 

mp : 142-144 °C

IR (Nujol) : 3430, 3350, 1750, 1690, 1640, 1525  $cm^{-1}$ 

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.23 (9H, s), 2.6-3.1 (6H, m), 3.9-4.25 (1H, m), 4.25-4.75 (2H, m), 5.03 (2H, s), 6.6-7.6 (11H, m), 7.14 (5H, s), 7.23 (5H, s), 7.73 (1H, br d, J = 8Hz), 8.51 (1H, br d, J = 8Hz), 10.64 (2H, s)

Elemental Analysis.

	Calculated for $C_{43}H_{45}N_5O_6$ :		
Found :	C 70.96, C 70.68,	H 6.23, H 6.17,	N 9.62 N 9.61



Example 49

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

5 Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(4)}$

To a solution of Boc-D-Trp(CHO)-OH (1.00 g), 2HCl·H-Phe-OCH<sub>2</sub>Py(4) (0.99 g) and HOBT (0.41 g) in DMF (25 ml) were added N,N-diisopropylethylamine (0.53 ml) and WSC (0.55 ml) under ice cooling. The mixture was stirred for an hour at this temperature and for additional 1.5 hours at room temperature. After  
 10 evaporation and extraction with ethyl acetate the organic layer was washed successively with water, 2% sodium hydrogencarbonate solution, water and saturated sodium chloride solution, and dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (40 g) and eluted with a mixture of chloroform and methanol (20:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with n-hexane and filtered. The powder was  
 15 dissolved in ethanol and reprecipitated with water, filtered and dried to give Boc-D-Trp(CHO)-Phe-OCH<sub>2</sub>Py(4) (1.29 g).

mp : 113-115 °C

IR (Nujol) : 3350, 1740, 1710, 1680, 1655, 1525 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.28 (9H, s), 2.6-3.3 (4H, m), 4.1-4.5 (1H, m), 4.5-4.9 (1H, m), 5.20 (2H, s), 6.92 (1H, br  
 20 d, J=9Hz), 7.1-7.9 (6H, m), 7.27 (5H, s), 7.9-8.4 (1H, m), 8.5-8.8 (3H, m), 9.4 (1H, broad)

Elemental Analysis.

	Calculated for C <sub>32</sub> H <sub>34</sub> N <sub>4</sub> O <sub>6</sub> :		
25 Found :	C 67.35, C 67.02,	H 6.01, H 5.98,	N 9.82 N 9.78

30 Example 50

Starting Compound :  $\text{Boc-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(4)}$

35 Object Compound :  $\text{Boc-Gln-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(4)·HCl}$

To a solution of Boc-Gln-D-Trp(CHO)-Phe-OCH<sub>2</sub>Py(4) (0.27 g) in a mixture of THF (25 ml) and DMF (5 ml) was added 4N-HCl/DOX (0.1 ml). After evaporation, the residue was pulverized with diethyl ether. The powder was filtered, washed with diisopropyl ether and dried to give Boc-Gln-D-Trp(CHO)-Phe-OCH<sub>2</sub>Py(4)-  
 40 ·HCl (0.24 g).

mp : ~160 °C

IR (Nujol) : 3300 (broad), 1750, 1710-1640 1530-1500 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.31 (9H, s), 1.5-2.1 (4H, m), 2.7-3.2 (4H, m), 3.8-4.1 (1H, m), 4.6 (10H, broad, overlapped with HOD), 5.42 (2H, s), 6.7-7.0 (2H, m), 7.0-7.8 (6H, m), 7.28 (5H, s), 7.89 (2H, d, J=6Hz), 8.0-  
 45 8.3 (2H, m), 8.89 (2H, d, J=6Hz), 9.3 (1H, broad)

Example 51

50 The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 8.

(1)

55 Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-OH}$

Object Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OCH}_2\text{Py(3)}$

mp : 144-145 °C

IR (Nujol) : 3410, 1720, 1690, 1650, 1545, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 2.5-3.2 (4H, m), 4.0-4.4 (1H, m), 4.4-4.8 (1H, m), 5.14 (2H, s), 6.80 (1H, br d,  $J=8\text{Hz}$ ), 7.0-7.5 (4H, m), 7.18 (5H, s), 7.5-7.8 (2H, m), 8.1 (1H, broad), 8.4-8.7 (3H, m), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{32}\text{H}_{34}\text{N}_4\text{O}_6$ :		
Found :	C 67.35, C 67.49,	H 6.01, H 6.02,	N 9.82 N 9.75

(2)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -OH

Object Compound : Boc-D-Trp-Phe-N $\begin{array}{l} \text{CHO} \\ | \\ \text{Et} \\ \text{Bzl(o-F)} \end{array}$

mp : 69-79 °C

IR (Nujol) : 3300, 1710, 1630  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.95 (t,  $J=6\text{Hz}$ ) and 1.01 (t,  $J=6\text{Hz}$ ) (3H), 1.25 (9H, s), 2.5-3.1 (4H, m), 3.1-3.6 (2H, m), 4.0-5.2 (4H, m), 6.7-6.9 (1H, m), 6.9-7.9 (13H, m), 8.1 (1H, br s), 8.60 (1H, br d,  $J=9\text{Hz}$ ), 9.0-9.7 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{35}\text{H}_{39}\text{FN}_4\text{O}_5 \cdot 1/2\text{H}_2\text{O}$ :		
Found :	C 67.40, C 67.28,	H 6.46, H 6.56,	N 8.98 N 8.74

(3)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -OH

Object Compound : Boc-D-Trp-Phe-N $\begin{array}{l} \text{CHO} \\ | \\ (\text{CH}_2)_2\text{OZ} \\ \text{Bzl} \end{array}$

mp : -70 °C

IR (Nujol) : 3300, 1745, 1710, 1635  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.24 (9H, s), 2.5-3.1 (4H, m), 3.2-3.6 (2H, m), 3.9-5.1 (6H, m), 5.09 (s) and 5.12 (s) (2H), 6.6-6.9 (1H, m), 6.9-7.55 (13H, m), 7.33 (5H, s), 7.55-7.8 (1H, m), 7.9-8.2 (1H, m), 8.4-8.8 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for $C_{43}H_{46}N_4O_8$ :		
Found :	C 69.15,	H 6.21,	N 7.50
	C 68.91,	H 6.07,	N 7.37

Example 52

The following object compounds were obtained from the corresponding starting compounds according to similar manner to those of Example 4 and Example 13, successively.

(1)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-OCH<sub>2</sub>Py(3)

Object Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-OCH<sub>2</sub>Py(3)

mp : 143-145 °C

IR (Nujol) : 3330, 1735, 1715, 1690, 1645, 1550, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 0.84 (3H, d, J = 6Hz), 1.34 (9H, s), 2.6-3.2 (4H, m), 3.6-4.0 (2H, m), 4.3-4.8 (3H, m), 5.11 (2H, s), 6.31 (1H, br d, J = 7Hz), 7.0-7.7 (6H, m), 7.17 (5H, s), 7.8-8.3 (2H, m), 8.4-8.7 (3H, m), 8.9-9.6 (1H, broad)

Elemental Analysis.

	Calculated for $C_{36}H_{41}N_5O_8$ :		
Found :	C 64.37,	H 6.15,	N 10.43
	C 64.15,	H 6.01,	N 10.37

(2)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-N $\begin{array}{l} \text{Me} \\ \text{Ph} \end{array}$

Object Compound : Boc-Thr-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-N $\begin{array}{l} \text{Me} \\ \text{Ph} \end{array}$

mp : 130-133 °C

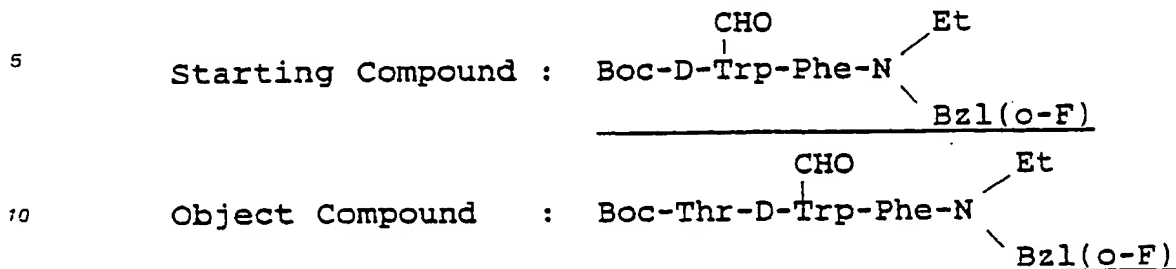
IR (Nujol) : 3330, 1710, 1690, 1650, 1630, 1590 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 0.81 (3H, d, J = 6Hz), 1.34 (9H, s), 2.5-3.1 (4H, m), 3.12 (3H, s), 3.6-4.0 (2H, m), 4.3-4.8 (3H, m), 6.22 (1H, br d, J = 9Hz), 6.6-6.9 (2H, m), 6.9-7.6 (12H, m), 7.88 (1H, br d, J = 9Hz), 8.0 (1H, broad), 8.47 (1H, br d, J = 9Hz), 9.1 (1H, broad)

Elemental Analysis.

	Calculated for $C_{37}H_{43}N_5O_7 \cdot 1/3H_2O$ :		
Found :	C 65.76,	H 6.51,	N 10.36
	C 65.89,	H 6.21,	N 10.38

(3)

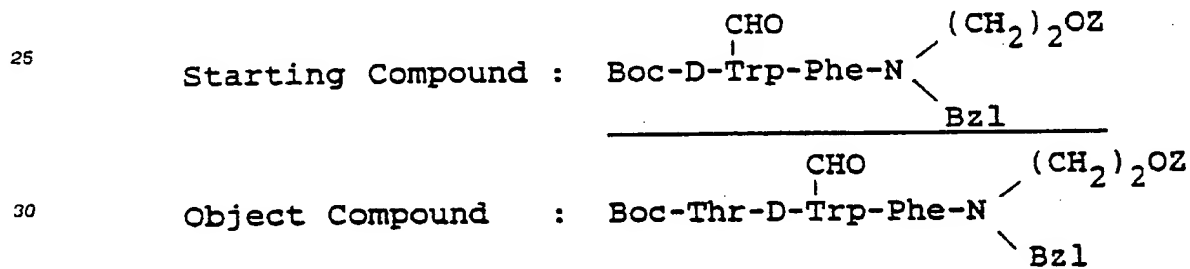


15 mp : 80-103 °C

IR (Nujol) : 3300, 1710, 1640, 1520 (broad), 1490  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.7-1.1 (6H, m), 1.33 (9H, s), 2.5-3.1 (4H, m), 3.1-3.5 (2H, m), 3.5-4.0 (2H, m), 4.2-5.1 (5H, m), 6.0-6.4 (1H, m), 6.8-7.7 (13H, m), 7.8-8.3 (2H, m), 8.5-8.8 (1H, m), 9.2 (1H, broad)

20

(4)

35 IR (Nujol) : 3300, 1745, 1710, 1640  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.83 (3H, d,  $J = 6\text{Hz}$ ), 1.33 (9H, s), 2.5-3.1 (4H, m), 3.2-4.0 (4H, m), 4.13 (2H, br s), 4.4-5.2 (5H, m), 5.10 (s) and 5.13 (s) (2H), 6.25 (1H, br d,  $J = 7\text{Hz}$ ), 6.9-7.7 (14H, m), 7.35 (5H, s), 7.7-8.3 (2H, m), 8.4-8.8 (1H, m), 9.2 (1H, broad)

Elemental Analysis.

40

Calculated for $\text{C}_{47}\text{H}_{53}\text{N}_5\text{O}_{10} \cdot 1/2\text{H}_2\text{O}$ :			
Found :	C 65.87,	H 6.35,	N 8.17
	C 65.84,	H 6.33,	N 8.00

45

Example 53

50

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 3.

55 (1)



Object Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-NHBzl

mp : 190-191 °C

IR (Nujol) : 3310, 1700, 1685, 1640, 1550, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 1.27 (9H, s), 2.6-3.1 (4H, m), 4.1-4.8 (2H, m), 4.35 (2H, d, J=6Hz), 6.92 (1H, br d, J=9Hz), 7.0-7.8 (14H, m), 8.2 (1H, broad), 8.47 (2H, br d, J=9Hz), 9.4 (1H, broad)  
 Elemental Analysis.

	Calculated for C <sub>33</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> :		
Found :	C 69.70, C 70.11,	H 6.38, H 6.41,	N 9.85 N 9.84

(2)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -OH

Object Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-N $\begin{array}{l} \text{Me} \\ \text{Ph} \end{array}$

mp : -102 °C

IR (Nujol) : 3300, 1710, 1640, 1595, 1495 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 1.27 (9H, s), 2.5-3.1 (4H, m), 3.16 (3H, s), 4.1-4.7 (2H, m), 6.6-7.0 (3H, m), 7.0-7.8 (12H, m), 8.15 (1H, br s), 8.46 (1H, br d, J=9Hz), 9.3 (1H, broad)

Elemental Analysis.

	Calculated for C <sub>33</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> • H <sub>2</sub> O :		
Found :	C 67.56, C 67.67,	H 6.53, H 6.60,	N 9.55 N 9.18

(3)

Starting Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -OH

Object Compound : Boc-D- $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-NHPh

mp : 213-215 °C

IR (Nujol) : 3310, 1695, 1650, 1600, 1530, 1510 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 1.25 (9H, s), 2.5-3.3 (4H, m), 4.1-4.5 (1H, m), 4.5-5.0 (1H, m), 6.7-7.0 (1H, m), 7.0-7.8 (14H, m), 8.1 (1H, broad), 8.53 (1H, d, J=8Hz), 9.3 (1H, broad), 9.95 (1H, s)  
 Elemental Analysis.

	Calculated for C <sub>32</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> :		
Found :	C 69.30, C 69.35,	H 6.18, H 6.33,	N 10.10 N 9.99

#### Example 54

The following object compounds were obtained from the corresponding starting compounds according

to a similar manner to that of Example 4.

(1)

5 Starting Compound : Boc-D-  $\begin{array}{c} \text{CH} \\ | \\ \text{Trp} \end{array}$  -Phe-NHBzl

Object Compound : HCl·H-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-NHBzl

IR (Nujol) : 3250 (broad), 1710, 1690, 1655  $\text{cm}^{-1}$

10 NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.5-3.3 (4H, m), 3.9-4.3 (1H, m), 4.30 (2H, d,  $J=6\text{Hz}$ ), 4.4-4.9 (1H, m), 7.0-7.5 (12H, m), 7.5-7.8 (2H, m), 8.0-8.3 (1H, broad), 8.36 (3H, br s), 8.88 (1H, br t,  $J=6\text{Hz}$ ), 9.27 (1H, d,  $J=9\text{Hz}$ ), 9.4 (1H, broad)

(2)

15 Starting Compound : Boc-D-Trp-Phe-NH<sub>2</sub>

Object Compound : HCl·H-D-Trp-Phe-NH<sub>2</sub>

mp : 222-228° C (dec.)

IR (Nujol) : 3400, 1675, 1610, 1570, 1500  $\text{cm}^{-1}$

20 NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.5-3.3 (4H, m), 3.8-4.1 (1H, m), 4.3-4.7 (1H, m), 6.8-7.4 (10H, m), 7.4-7.7 (2H, m), 7.94 (3H, s), 8.90 (1H, d,  $J=9\text{Hz}$ ), 10.88 (1H, s)

Elemental Analysis.

25

	Calculated for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_2 \cdot \text{HCl}$ :			
	C 62.09,	H 5.99,	N 14.48,	Cl 9.16
Found :	C 61.89,	H 5.93,	N 14.37,	Cl 9.37

30

### Example 55

The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 13.

35

(1)

Starting Compound : HCl·H-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-NHBzl

40 Object Compound : Boc-Gln-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-NHBzl

mp : -206° C (dec.)

IR (Nujol) : 3300, 1705, 1690, 1660, 1640, 1545  $\text{cm}^{-1}$

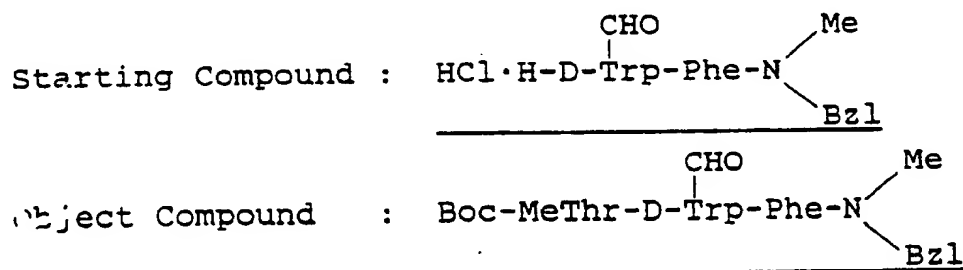
NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.30 (9H, s), 1.5-2.2 (4H, m), 2.6-3.1 (4H, m), 3.7-4.2 (1H, m), 4.31 (2H, d,  $J=6\text{Hz}$ ), 4.5-4.9 (2H, m), 6.6-6.9 (2H, m), 7.1-7.8 (15H, m), 7.8-8.3 (2H, m), 8.4-8.7 (2H, m), 9.3 (1H, broad)

45 Elemental Analysis.

50

	Calculated for $\text{C}_{38}\text{H}_{44}\text{N}_6\text{O}_7 \cdot 1/3\text{H}_2\text{O}$ :		
	C 64.94,	H 6.41,	N 11.96
Found :	C 64.93,	H 6.64,	N 11.89

55 (2)

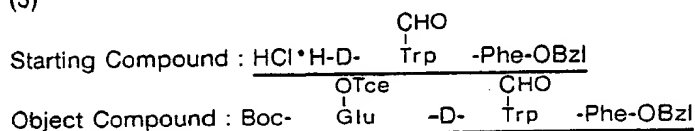


mp : 75-80° C

IR (Nujol) : 3420, 3300, 1710-1640  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.6-1.0 (3H, m), 1.35 (9H, s), 2.6-3.1 (4H, m), 2.73 (3H, s), 2.78 (s) and 2.85 (s) (3H), 3.6-5.2 (7H, m), 6.9-7.8 (14H, m), 7.8-8.2 (2H, m), 8.65 (1H, broad), 9.2 (1H, broad)

(3)

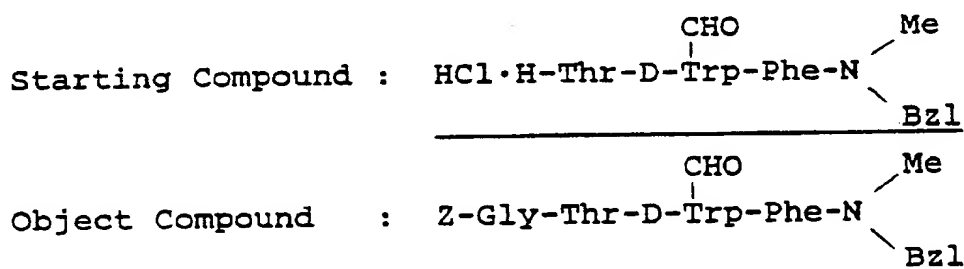


mp : 147-155° C

IR (Nujol) : 3330, 1720, 1690, 1645, 1540, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.32 (9H, s), 1.4-1.9 (2H, m), 1.9-2.4 (2H, m), 2.6-3.2 (4H, m), 3.8-4.3 (1H, m), 4.4-4.9 (2H, m), 4.83 (2H, s), 5.13 (2H, s), 6.7-7.0 (1H, m), 7.2-7.5 (3H, m), 7.25 (5H, s), 7.36 (5H, s), 7.5-7.8 (1H, m), 7.9-8.3 (2H, m), 8.6-8.9 (1H, m), 9.3 (1H, broad)

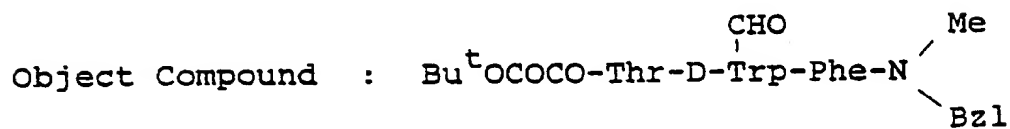
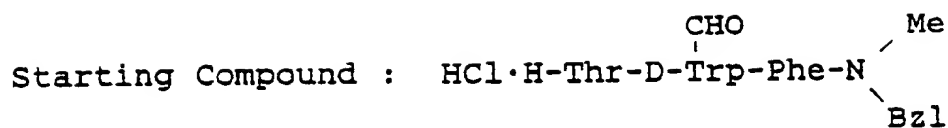
(4)



IR (Nujol) : 3300, 1710, 1640 (sh), 1630, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 2.6-3.1 (4H, m), 2.77 and 2.84 (3H, s), 3.70 (2H, d,  $J=6\text{Hz}$ ), 3.8 (1H, m), 4.1 (1H, m), 4.3-5.0 (5H, m), 4.92 (2H, s), 6.9-7.7 (15H, m), 7.27 (5H, s), 8.0 (2H, m), 8.6 (1H, t,  $J=6\text{Hz}$ ), 9.15 (1H, br s)

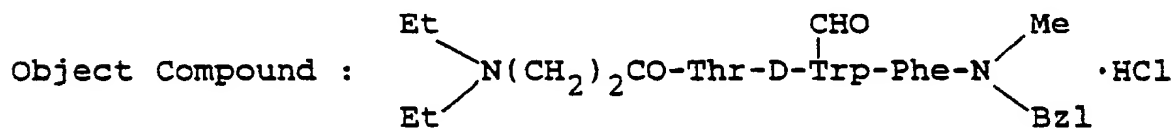
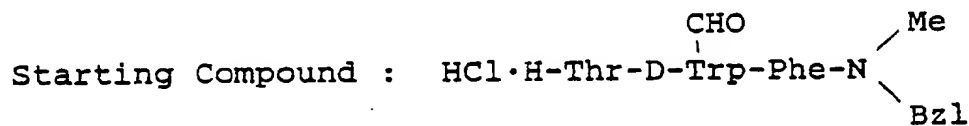
(5)



IR (Nujol) : 3300, 1710, 1660, 1630  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 1.09 (3H, d,  $J=6\text{Hz}$ ), 1.48 (9H, s), 2.16 (1H, s), 2.67 and 2.77 (3H, s), 2.87 (2H, m), 3.15 (2H, m), 4.2-4.4 (4H, m), 4.6-5.1 (2H, m), 6.9-7.35 (14H, m), 7.45-7.6 (2H, m), 7.85 (1H, d,  $J=7\text{Hz}$ ), 8.25 (1H, br), 9.0 (1H, br)

(6)



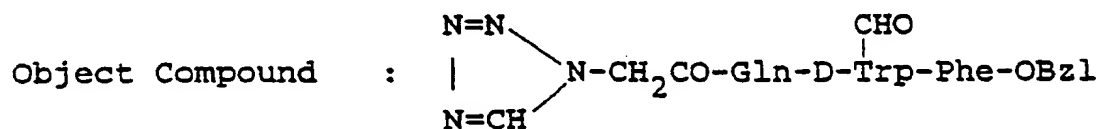
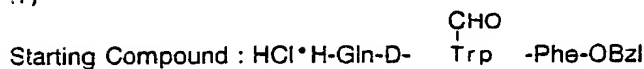
IR (Nujol) : 3300, 1710, 1660 (sh), 1640  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 1.17 (6H, t,  $J=7\text{Hz}$ ), 2.77 and 2.83 (3H, s), 2.6-3.3 (12H, m), 3.77 (1H, m), 4.0-4.4 (3H, m), 4.5-4.8 (2H, m), 4.95 (1H, m), 7.0-7.4 (13H, m), 7.45-7.8 (2H, m), 8.0-8.3 (2H, m), 8.65 (1H, m), 9.3 (1H, br), 10.45 (1H, br)

Elemental Analysis.

	Calculated for $\text{C}_{40}\text{H}_{50}\text{N}_6\text{O}_6 \cdot \text{HCl} \cdot 2.5\text{H}_2\text{O}$ :		
Found :	C 62.04, C 61.44,	H 6.90, H 6.89,	N 10.35 N 10.86

(7)





mp : 225-227° C (dec.)

IR (Nujol) : 3450, 3300, 1730 (sh), 1710, 1660, 1640, 1650 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.5-2.15 (4H, m), 2.8 (2H, m), 3.1 (2H, m), 4.4 (1H, m), 4.7 (2H, m), 5.17 (2H, s), 5.30 (2H, s), 6.73 (1H, br), 7.27 (5H, s), 7.37 (5H, s), 7.2-7.6 (4H, m), 7.7 (1H, m), 8.2 (1H, m), 8.37 (1H, d,

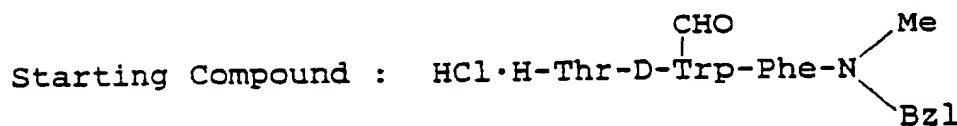
5 J=9Hz), 8.7 (2H, m), 9.27 (1H, br), 9.33 (1H, s)

Elemental Analysis.

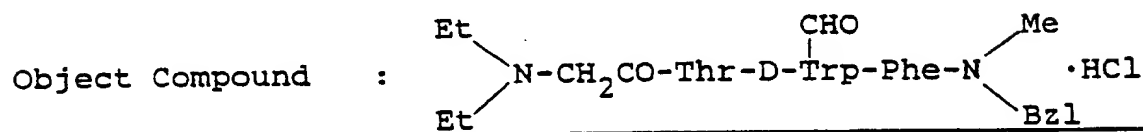
10

	Calculated for C <sub>36</sub> H <sub>37</sub> N <sub>3</sub> O <sub>6</sub> :		
Found :	C 59.84, C 59.37(59.29),	H 5.92, H 5.38(5.32),	N 17.89 N 17.47(17.40)

15 (8)



20



25

NMR (DMSO-d<sub>6</sub>, δ) : 0.83 (3H, d, J=6Hz), 1.13 (6H, t, J=7Hz), 2.87 (3H, s), 2.78 (2H, br), 2.90-3.0 (2H, m), 3.80 (1H, m), 3.97 (2H, s), 4.20 (1H, m), 4.3-5.0 (4H, m), 7.0-7.42 (13H, m), 7.5-7.8 (2H, m), 8.2 (2H, m), 8.7 (1H, m), 9.3 (1H, br), 9.9 (1H, br)

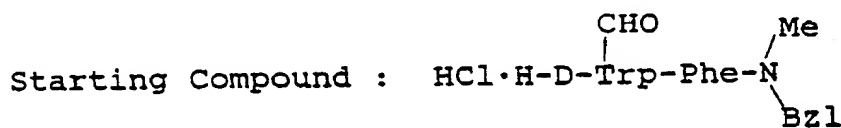
30

Elemental Analysis.

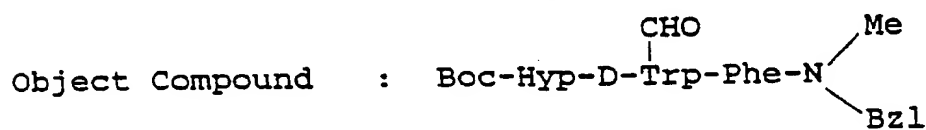
35

	Calculated for C <sub>39</sub> H <sub>48</sub> N <sub>6</sub> O <sub>6</sub> · HCl :			
Found :	C 63.88, C 59.93,	H 6.73, H 6.73,	N 11.46, N 10.81,	Cl 4.83 Cl 4.73

40 (9)



45



50

IR (Nujol) : 3300, 1710, 1690, 1670, 1630 cm<sup>-1</sup>

55 NMR (DMSO-d<sub>6</sub>, δ) : 1.13, 1.20 and 1.33 (9H, s), 2.6-3.0 (9H, m), 3.23 (2H, m), 3.9-4.2 (2H, m), 4.3-5.1 (5H, m), 6.9-7.5 (14H, m), 7.65 (1H, m), 7.9-8.3 (2H, m), 8.8 (1H, m), 9.3 (1H, br)

(10)

Starting Compound :  $\text{HCl} \cdot \text{H-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-OBzl}$

Object Compound :  $\begin{array}{c} \text{Bzl} \\ \diagup \\ \text{N-CH}_2\text{CO-D-Trp-Phe-OBzl} \\ \diagdown \\ \text{Boc} \end{array}$

mp : 109-110° C

IR (Nujol) : 3300, 1740, 1710, 1690, 1640  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.37 (9H, br s), 2.81 (2H, m), 3.07 (2H, m), 3.69 (2H, m), 4.28 (2H, m), 4.5-4.9 (2H, m), 5.14 (2H, s), 7.24 (5H, s), 7.38 (5H, s), 7.05-7.5 (9H, m), 7.66 (1H, m), 8.12 (1H, m), 8.78 (1H, d, J=8Hz), 9.31 (1H, br s)

Elemental Analysis.

	Calculated for $\text{C}_{42}\text{H}_{44}\text{N}_4\text{O}_7$ :		
Found :	C 70.37, C 69.42,	H 6.19, H 6.39,	N 7.82 N 7.58

#### Example 56

Starting Compound :  $\text{Boc-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-NHPh}$

Object Compound :  $\text{Boc-Thr-D-} \begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array} \text{-Phe-NHPh}$  and  $\text{Boc-Thr-D-Trp-Phe-NHPh}$

A mixture of Boc-D-Trp(CHO)-Phe-NHPh (0.93 g) in 4N-HCl/DOX (15 ml) was stirred for 3 hours. After evaporation, the residue was pulverized with diethyl ether, filtered washed with diethyl ether and dried. The residual powder (0.78 g) of  $\text{HCl} \cdot \text{H-D-Trp(CHO)-Phe-NHPh}$ . Boc-Thr-OH (0.35 g) and HOBT (0.21 g) was dissolved in DMF (15 ml). To the solution was added WSC (0.29 ml) under ice-cooling and the mixture was stirred at room temperature. After stirring for 3, 4 and 5 hours, triethylamine (0.04 ml) was added respectively. Stirring was continued for further an hour. After evaporation, the residue was crystallized with 2% hydrochloric acid. The crystals were filtered, washed with water, 2% sodium hydrogen carbonate (twice) and water. The resultant crystals were subjected to column chromatography on silica gel (100 g) and eluted with a mixture of chloroform and methanol (50:1 to 30:1, gradient elution). The fractions containing less polar compound were combined and evaporated. The residue was pulverized with diisopropyl ether, filtered and dried to give

Boc-Thr-D-Trp(CHO)-Phe-NHPh (0.10 g).

mp : 158-160° C

IR (Nujol) : 3300, 1700, 1690, 1640, 1545  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.81 (3H, d, J=6Hz), 1.32 (9H, s), 2.6-3.3 (4H, m), 3.6-4.0 (2H, m), 4.4-4.8 (3H, m), 6.25 (1H, br d, J=9Hz), 6.9-7.7 (14H, m), 7.8-8.2 (2H, m), 8.55 (1H, br d, J=8Hz), 9.2 (1H, broad), 9.97 (1H, s)

The next fractions containing more polar compound on column chromatography were combined and evaporated. The residue was pulverized with diisopropyl ether, filtered and dried to give Boc-Thr-D-Trp-Phe-NHPh (0.45 g).

mp : 223-226° C

IR (Nujol) : 3450, 3340, 1700, 1655, 1650, 1535  $\text{cm}^{-1}$

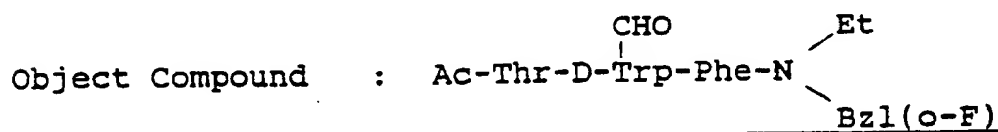
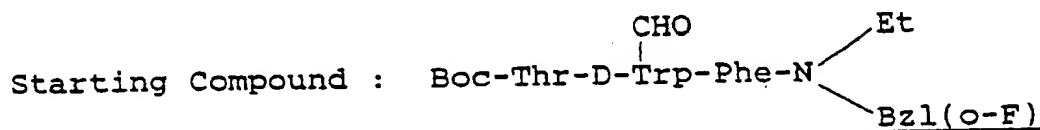
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.88 (3H, br d, J=6Hz), 1.32 (9H, s), 2.6-3.3 (4H, m), 3.6-4.0 (2H, m), 4.3-4.8 (3H, m), 6.26 (1H, br d, J=8Hz), 6.8-7.8 (15H, m), 7.92 (1H, br d, J=7Hz), 8.40 (1H, br d, J=8Hz), 9.79 (1H, s), 10.70 (1H, s)

Elemental Analysis:

	Calculated for $C_{35}H_{41}N_5O_6 \cdot 1/2H_2O$ :		
Found :	C 66.02,	H 6.65,	N 11.00
	C 66.28,	H 6.47,	N 11.03

### Example 57

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 23.

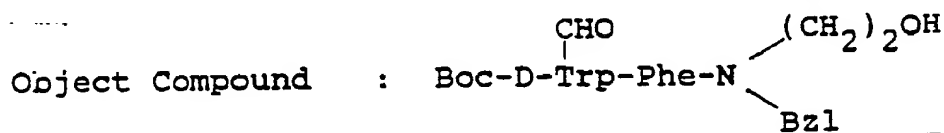
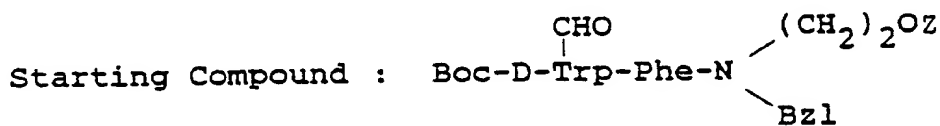


mp :  $-110^\circ\text{C}$  (dec.)

IR (Nujol) : 3310, 1710, 1640 (broad),  $1535\text{ cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.7-1.2 (6H, m), 1.83 (3H, s), 2.5-3.1 (4H, m), 3.1-3.5 (2H, m), 3.5-3.9 (1H, m), 3.9-4.2 (1H, m), 4.2-5.1 (5H, m), 6.9-7.8 (14H, m), 7.8-8.3 (2H, m), 8.5-8.8 (1H, m), 9.2 (1H, broad)

### Example 58



Boc-D-Trp(CHO)-Phe-N $((\text{CH}_2)_2\text{OZ})$ Bzl (0.75 g) was hydrogenated in ethanol (10 ml) with 10% palladium on carbon (0.15 g). The catalyst was filtered off and the filtrate was condensed under reduced pressure. The residue was subjected to column chromatography on silica gel (50 g) and eluted with chloroform and then a mixture of chloroform and methanol (50:1). The fractions containing the object compound was combined and evaporated. The residue was pulverized with n-hexane, filtered and dried to give Boc-D-Trp(CHO)-Phe-N $((\text{CH}_2)_2\text{OH})$ Bzl (0.57 g)

IR (Nujol) : 3300, 1710,  $1630\text{ cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.27 (9H, s), 2.5-3.1 (4H, m), 3.1-3.8 (4H, m), 4.0-5.3 (5H, m), 6.78 (1H, br d,  $J=8\text{Hz}$ ), 6.9-7.9 (13H, m), 7.9-8.3 (11H, m), 8.58 (1H, d,  $J=9\text{Hz}$ ), 9.3 (1H, broad)

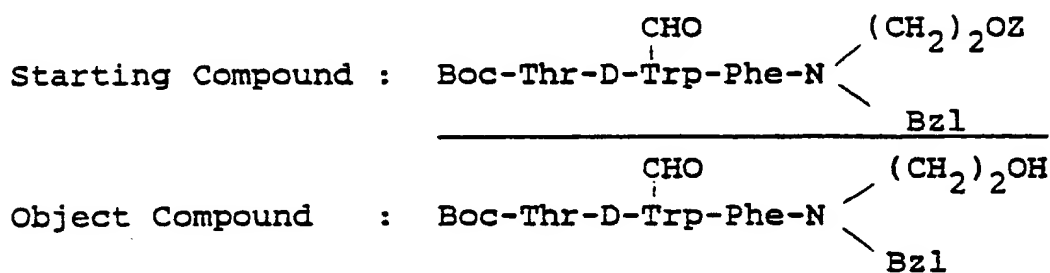
Elemental Analysis.

	Calculated for $C_{35}H_{40}N_4O_6 \cdot 1/2H_2O$ :		
Found :	C 67.62, C 68.00,	H 6.65, H 6.61,	N 9.01 N 8.75

### Example 59

The following object compound were obtained from the corresponding starting compounds according to a similar manner to that of Example 58.

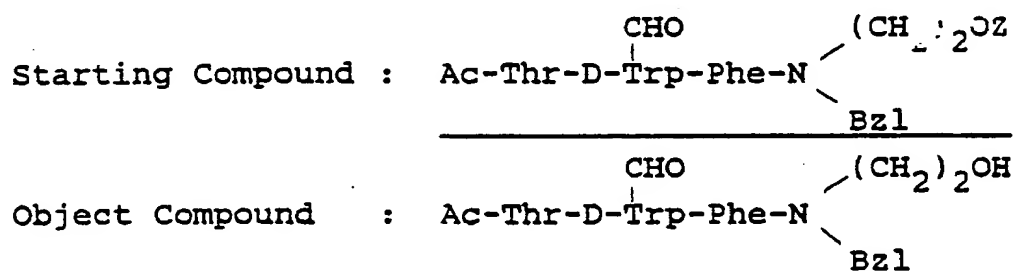
(1)



IR (Nujol) : 3300, 1705, 1635 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.83 (3H, d,  $J=5\text{Hz}$ ), 1.35 (9H, s), 2.5-4.0 (10H, m), 4.4-5.2 (6H, m), 6.1-6.4 (1H, m), 6.9-7.7 (14H, m), 7.7-8.2 (2H, m), 8.4-8.8 (1H, m), 9.15 (1H, broad)

(2)



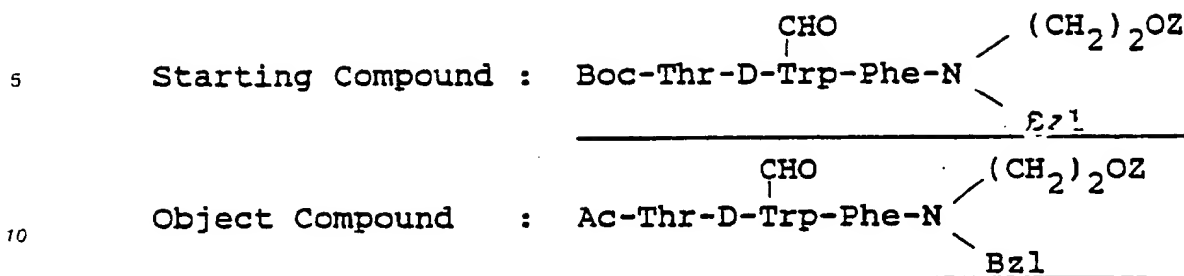
IR (Nujol) : 3300, 1635 (broad), 1545, 1525 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.77 (3H, d,  $J=6\text{Hz}$ ), 1.85 (3H, s), 2.6-3.9 (9H, m), 4.0-4.3 (1H, m), 4.4-5.3 (6H, m), 6.9-7.6 (13H, m), 7.6-7.9 (2H, m), 7.9-8.3 (2H, m), 8.66 (1H, d,  $J=9\text{Hz}$ ), 9.2 (1H, broad)

(3)



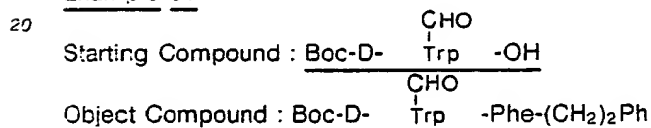
similar manner to that of Example 23.



IR (Nujol) : 3300, 1750, 1710, 1640, 1525, (broad)  $\text{cm}^{-1}$

15      NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.77 (3H, d,  $J=6\text{Hz}$ ), 1.84 (3H, s), 2.6-3.1 (4H, m), 3.2-4.3 (6H, m), 4.4-5.1 (5H, m), 5.12 (2H, s), 6.9-7.8 (15H, m), 7.34 (5H, s), 7.8-8.3 (2H, m), 8.4-8.8 (1H, m), 9.2 (1H, broad)

#### Example 61



25      To a solution of Boc-D-Trp(CHO)-OH (0.92 g) in methylene chloride (15 ml) were added NMM (0.28 ml) and isobutyl chloroformate (0.36 ml) successively at  $-15^\circ\text{C}$ , and the mixture was stirred for ten minutes. On the other hand, a solution of  $\text{HCl}\cdot\text{H-Phe-(CH}_2)_2\text{Ph}$  (0.80 g) in methylene chloride (15 ml) was cooled at  $-30^\circ\text{C}$  and thereto was added NMM (0.28 ml). This solution was added to the above mentioned mixture at  $-50^\circ\text{C}$ , and stirred for an hour at  $-50^\circ\text{C}$  and then stirred for 2 hours at room temperature. After evaporation and extraction with ethyl acetate, the organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogen carbonate solution, water, and saturated sodium chloride solution, and dried over magnesium sulfate. After evaporation, the residual white crystals were filtered and washed with n-hexane. The crystals were recrystallized from ethanol to give Boc-D-Trp(CHO)-Phe-(CH<sub>2</sub>)<sub>2</sub>Ph (1.16 g).

mp :  $171-172^\circ\text{C}$

35      IR (Nujol) : 3350, 1720, 1660, 1520  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.13 (9H, s), 2.5-3.2 (8H, m), 4.25 (1H, br q,  $J=7\text{Hz}$ ), 4.3-4.7 (1H, m), 6.6-7.7 (5H, m), 7.10(10H, s), 7.8-8.2 (1H, m), 8.58 (1H, d,  $J=9\text{Hz}$ ), 9.3 (1H, broad)

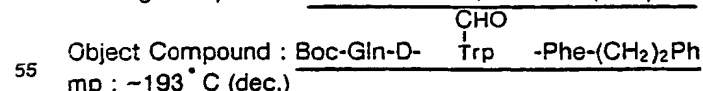
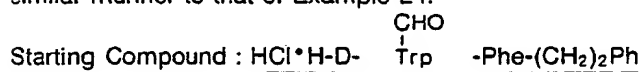
Elemental Analysis.

40

	Calculated for $\text{C}_{34}\text{H}_{37}\text{N}_3\text{O}_5$ :		
Found :	C 71.94, C 71.80,	H 6.57, H 6.58,	N 7.40 N 7.53

#### Example 62

50      The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 24.



IR (Nujol) : 3330, 1710, 1690, 1655, 1640, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.31 (9H, s), 1.4-2.1 (4H, m), 2.5-3.3 (8H, m), 3.7-4.1 (1H, m), 4.3-4.8 (2H, m), 6.6-6.9 (2H, m), 7.0-7.8 (5H, m), 7.18 (10H, s), 7.8-8.3 (2H, m), 8.3-8.7 (1H, m), 9.25 (1H, broad)

## Elemental Analysis.

5

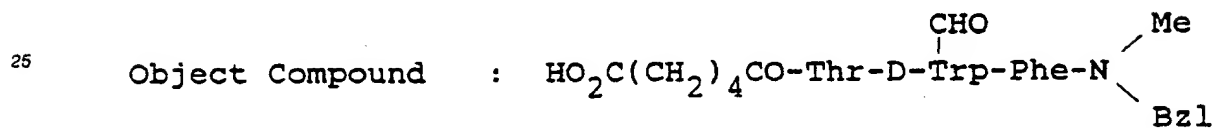
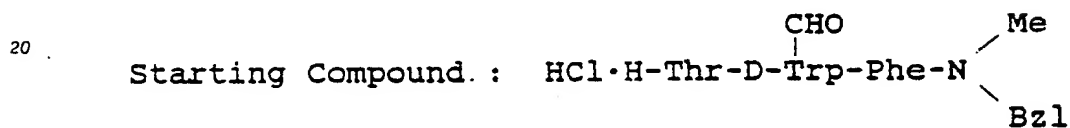
	Calculated for C <sub>39</sub> H <sub>45</sub> N <sub>5</sub> O <sub>7</sub> :		
Found :	C 67.32, C 67.14,	H 6.52, H 6.52,	N 10.06 N 10.03

10 Example 63

The following compounds were obtained from the compounding starting compounds according to a similar manner to that of Example 17.

15

(1)



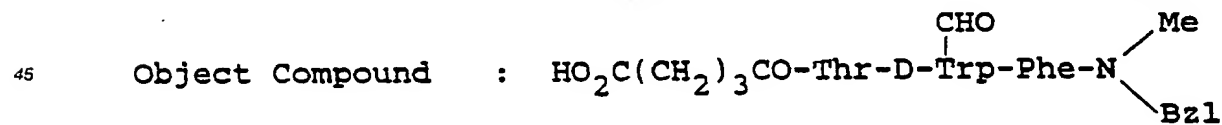
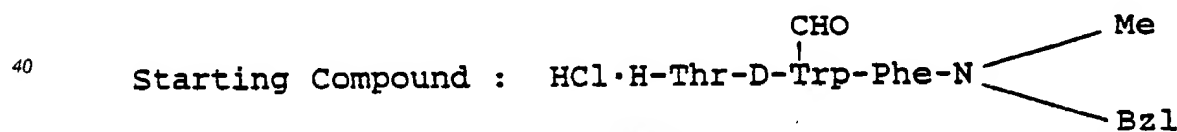
30 mp : 110-116° C

IR (Nujol) : 3300, 1710, 1640, 1540 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.81 (3H, d, J = 6Hz), 1.46 (4H, br s), 1.8-2.3 (4H, m), 2.6-3.2 (4H, m), 2.77 (s) and 2.83 (s) (3H), 3.6-4.0 (1H, m), 4.0-5.2 (6H, m), ca. 6.3 (1H, broad), 6.9-7.4 (12H, m), 7.4-7.8 (3H, m), 7.8-8.2 (2H, m), 8.4-8.8 (1H, m), 9.2 (1H, broad)

35

(2)



50 mp : ~145° C (dec.)

IR (Nujol) : 3300, 1710, 1635, 1540 (broad) cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.82 (3H, d, J = 6Hz), 1.5-1.9 (2H, m), 1.9-2.4 (4H, m), 2.6-3.2 (4H, m), 2.75 (s) and 2.82 (s) (3H), 3.7-4.0 (1H, m), 4.0-5.2 (7H, m), 6.9-7.4 (12H, m), 7.4-7.8 (3H, m), 7.9-8.3 (2H, m), 8.4-8.8 (1H, m), 9.3 (1H, broad)

55

(3)

Starting Compound :  $\text{HCl} \cdot \text{H-Thr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ \text{Me} \\ \text{Bzl} \end{matrix}$

Object Compound :  $\text{HO}_2\text{C}(\text{CH}_2)_2\text{CO-Thr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ \text{Me} \\ \text{Bzl} \end{matrix}$

mp :  $\sim 160^\circ \text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1640, 1540 (broad)  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.84 (3H, d,  $J=6\text{Hz}$ ), 2.35 (4H, s), 2.6-3.1 (7H, m), 3.7-5.1 (8H, m), 6.9-7.4 (12H, m), 7.4-7.9 (3H, m), 7.9-8.3 (2H, m), 8.6-8.9 (1H, m), 9.2 (1H, broad)

Elemental Analysis.

	Calculated for $\text{C}_{37}\text{H}_{41}\text{N}_5\text{O}_8 \cdot \text{H}_2\text{O}$		
	:		
Found :	C 63.33,	H 6.18,	N 9.89
	C 63.03,	H 5.90,	N 9.79

(4)

Starting Compound :  $\text{Ac-Thr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ \text{Me} \\ \text{Bzl} \end{matrix}$

Object Compound :  $\text{Ac-Thr-D-Trp-Phe-N} \begin{matrix} \text{CO}(\text{CH}_2)_2\text{CO}_2\text{H} \\ \text{CHO} \\ \text{Me} \\ \text{Bzl} \end{matrix}$

mp :  $\sim 135^\circ \text{C}$

IR (Nujol) : 3500, 3290, 1735, 1710, 1640, 1550  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.82 (3H, d,  $J=6\text{Hz}$ ), 1.82 (3H, s), 2.27 (4H, s), 2.86 (3H, s), 2.6-3.0 (4H, m), 4.30 and 4.53 (2H, ABq,  $J=15\text{Hz}$ ), 4.4-5.1 (4H, m), 6.9-7.6 (13H, m), 7.7 (1H, m), 7.90 (1H, d,  $J=7\text{Hz}$ ), 8.1 (1H, m), 8.22 (1H, d,  $J=7\text{Hz}$ ), 8.73 (1H, m), 9.28 (1H, br)

Elemental Analysis.

	Calculated for $\text{C}_{38}\text{H}_{43}\text{N}_5\text{O}_7 \cdot \text{H}_2\text{O}$		
	:		
Found :	C 62.98,	H 6.10,	N 9.42
	C 62.98,	H 6.20,	N 9.48

(5)

Starting Compound :  $\text{HCl} \cdot \text{H-Gln-D-Trp-Phe-OBzl}$



Object Compound :  $\text{HO}_2\text{C}(\text{CH}_2)_2\text{CO-Gln-D-}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-OBzl}$   
 mp: 229-230 °C (dec.)

IR (Nujol) : 3400, 3280, 1725, 1710, 1660, 1640, 1550  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.47-2.1 (4H, m), 2.40 (4H, s), 2.86 (2H, m), 3.04 (2H, m), 4.20 (1H, m), 4.63 (2H, m),  
 5.13 (2H, s), 6.73 (1H, br), 7.28 (5H, s), 7.37 (5H, s), 7.1-7.5 (4H, m), 7.6 (1H, m), 8.1 (3H, m), 8.73 (1H, d,  
 J = 7Hz), 9.3 (1H, br)

(6)

Starting Compound :  $\text{HCl} \cdot \text{H-Thr-D-}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-N}$   $\begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$

Object Compound :  $\text{O} \begin{array}{c} \text{N-CO-Thr-D-} \end{array}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-N}$   $\begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$

IR (Nujol) : 3400, 3280, 1710, 1660 (sh), 1640, (sh), 1630, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, t, J = 6Hz), 2.77 (2H, m), 2.83 (3H, s), 2.83 (2H, m), 3.28 (4H, s), 3.50 (4H, br  
 s), 3.65-4.1 (2H, m), 4.2-5.1 (5H, m), 6.12 (1H, d, J = 7Hz), 6.95-7.4 (13H, m), 7.4-7.6 (2H, m), 8.1 (6H, m),  
 8.6 (1H, m), 9.25 (1H, br s)

(7)

Starting Compound :  $\text{HCl} \cdot \text{H-Thr-D-}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-N}$   $\begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$

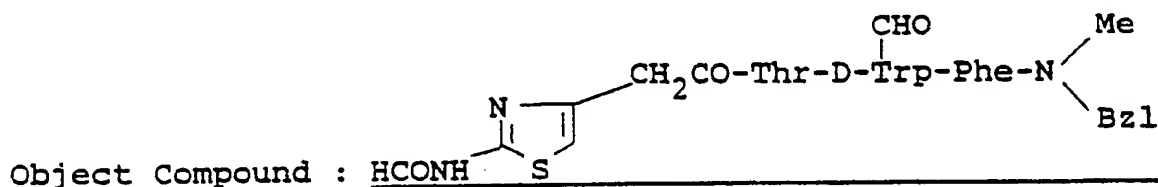
Object Compound :  $\text{Bu}^t\text{NHCO-Thr-D-}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-N}$   $\begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$

IR (Nujol) : 3360, 3220, 1710, 1650, 1630, 1550  $\text{cm}^{-1}$

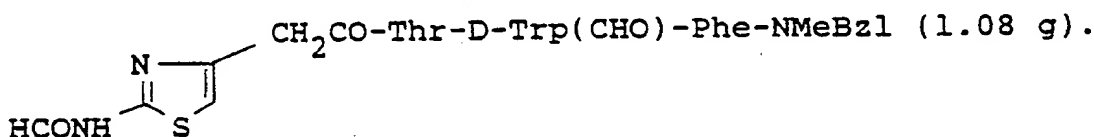
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.78 (3H, d, J = 6Hz), 1.20 (9H, s), 2.83 (3H, s), 2.6-3.15 (4H, m), 3.6-4.05 (2H, m), 4.30  
 and 4.63 (2H, ABq, J = 15Hz), 4.5-5.2 (3H, m), 5.90 (1H, d, J = 7Hz), 6.14 (1H, s), 6.9-7.7 (15H, m), 7.86 (1H,  
 m), 8.13 (1H, m), 8.66 (1H, m), 9.23 (1H, br s)

Example 64

Starting Compound :  $\text{HCl} \cdot \text{H-Thr-D-}$   $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$   $\text{-Phe-N}$   $\begin{array}{l} \text{Me} \\ \text{Bzl} \end{array}$



To a solution of DMF (0.17 ml) in ethyl acetate (0.68 ml) was added phosphorus oxychloride (0.20 ml) at  $-10^{\circ}\text{C}$ . The mixture was stirred for 25 minutes. 2-Formamidothiazol-4-ylacetic acid (0.37 g) and ethyl acetate (0.68 ml) were added and the mixture was stirred for an hour (mixture A). On the other hand, to the mixture of  $\text{HCl}\cdot\text{H-Thr-D-Trp(CHO)-Phe-NMeBzl}$  (1.24 g) in ethyl acetate (20 ml) was added bis-(trimethylsilyl)-acetamide (3.0 ml). After stirring for an hour at room temperature, the mixture was cooled at  $-15^{\circ}\text{C}$ . To the mixture was added the mixture A and stirred for 1.5 hours at  $-15^{\circ}\text{C}$ . Water (15 ml) was added and the mixture was stirred for 20 minutes at room temperature. The organic layer was separated and washed with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate, water and saturated sodium chloride solution and dried over magnesium sulfate. After evaporation the residue was subjected to column chromatography on silica gel (100 g) and eluted with a mixture of chloroform and methanol (30:1). The fractions containing the object compound were combined and evaporated. The residue was pulverized with diisopropyl ether, filtered and dried to give



mp :  $-130^{\circ}\text{C}$  (dec.)

IR (Nujol): 3300, 1710-1640, 1545-1510  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.82 (3H, d,  $J=6\text{Hz}$ ), 2.6-3.2 4H, m), 2.77 (s) and 2.84 (s) (3H), 3.57 (2H, s), 3.7-5.1 (7H, m), 6.88 (1H, s), 6.9-7.7 (14H, m), 7.7-8.4 (4H, m), 8.6-8.9 (1H, m), 9.1 (1H, broad), 12.0 (1H, broad) Elemental Analysis.

	Calculated for $\text{C}_{39}\text{H}_{41}\text{N}_7\text{O}_7\cdot 5/2\text{H}_2\text{O}$ :		
Found :	C 58.78, C 58.74,	H 5.82, H 5.46,	N 12.30 N 11.97

#### Example 65

The following compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 15.

(1)

Starting Compound :  $\text{Boc-MeThr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ | \\ \text{Me} \\ \text{Bzl} \end{matrix}$

Object Compound :  $\text{HCl} \cdot \text{H-MeThr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ | \\ \text{Me} \\ \text{Bzl} \end{matrix}$

mp :  $\sim 148^\circ \text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1675, 1635, 1550  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.64 (3H, d,  $J=6\text{Hz}$ ), 2.34 (3H, s), 2.6-3.1 (4H, m), 2.77 (s) and 2.86 (s) (3H), 3.4-3.8 (2H, m), 4.2-5.2 (4H, m), 5.5-5.7 (1H, m), 6.9-7.5 (12H, M), 7.59 (1H, s), 7.7-7.9 (1H, m), 7.9-8.2 (1H, m), 8.7-9.1 (4H, m), 9.3 (1H, broad)

(2)

Starting Compound: Boc-Gln-D-Trp-Phe-NH<sub>2</sub>

Object Compound : H-Gln-D-Trp-Phe-NH<sub>2</sub>

mp :  $\sim 269^\circ \text{C}$  (dec.)

IR (Nujol) : 3300, 1670 (broad), 1640, 1535  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.3-2.2 (6H, m), 2.6-3.4 (5H, m), 4.2-4.6 (2H, m), 6.6 (1H, br s), 6.7-7.5 (3H, m), 7.9 (1H, broad), 8.24 (1H, d,  $J=9\text{Hz}$ ), 10.64 (1H, s).

Elemental Analysis.

	Calculated for $\text{C}_{25}\text{H}_{30}\text{N}_6\text{O}_4 \cdot 1/4\text{H}_2\text{O}$ :		
Found :	C 62.16, C 62.23,	H 6.36, H 6.19,	N 17.40 N 17.24

(3)

Starting Compound : Boc-D-Trp-D-Trp-Phe-OBzl

Object Compound : HCl  $\cdot$  H-D-Trp-D-Trp-Phe-OBzl

(4)

Starting Compound :  $\text{Bu}^t\text{O}_2\text{C-CO-Thr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ | \\ \text{Me} \\ \text{Bzl} \end{matrix}$

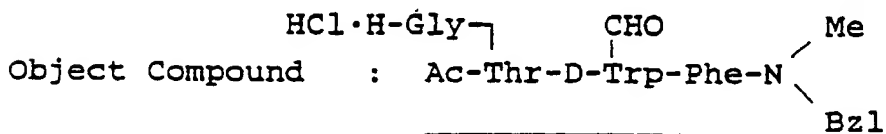
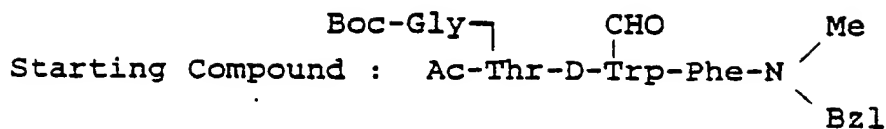
Object Compound :  $\text{HO}_2\text{CCO-Thr-D-Trp-Phe-N} \begin{matrix} \text{CHO} \\ | \\ \text{Me} \\ \text{Bzl} \end{matrix}$

mp :  $137^\circ \text{C}$  (dec.)

IR (Nujol) : 3300, 1730 (sh), 1710, 1630  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 2.77, 2.87 (s), and 2.5-3.0 (m) (7H), 3.87 (1H, m), 4.1-4.25 (1H, m), 4.35-5.1 (5H, m), 6.9-7.4 (9H, m), 7.2 (5H, s), 7.6 (1H, m), 7.95-8.3 (3H, m), 8.6 (1H, m), 9.2 (1H, br)

(5)

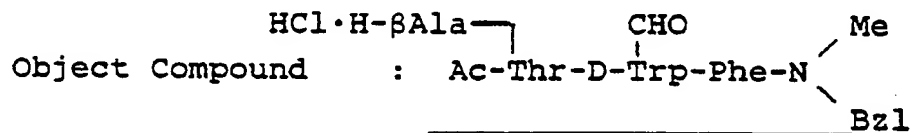
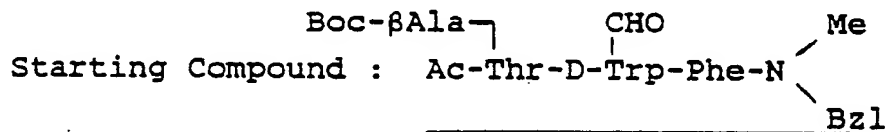


mp :  $-120^{\circ}\text{C}$

IR (Nujol) : 3280, 1760, 1710 (h), 1695 (sh), 1670, 1640  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.87 (3H, d,  $J=6\text{Hz}$ ), 1.87 (3H, s), 2.83 (3H, s), 2.6-3.0 (4H, m), 3.67 (2H, s), 4.28 and 4.63 (2H, ABq,  $J=15\text{Hz}$ ), 4.95 (2H, m), 4.5 (2H, m), 6.9-7.3 (13H, m), 7.47 (1H, m), 7.67 (1H, m), 8.02 (1H, d,  $J=7\text{Hz}$ ), 8.29 (4H, br), 8.70 (1H, d,  $J=7\text{Hz}$ ), 9.25 (1H, br)

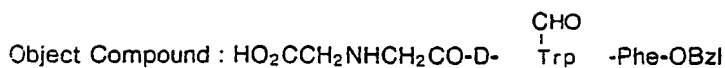
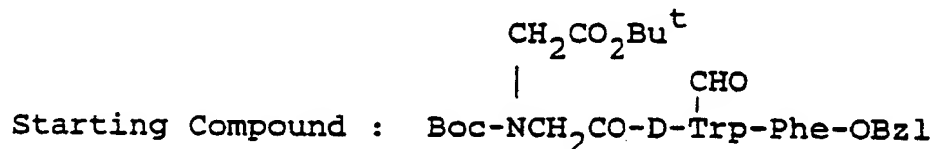
(6)



IR (Nujol) : 3250, 1740, 1710, 1660 (sh), 1640  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.87 (3H, d,  $J=7\text{Hz}$ ), 1.87 (3H, s), 2.56 (2H, t,  $J=7\text{Hz}$ ), 2.87 (3H, s), 2.7-3.15 (4H, m), 4.30 and 4.63 (2H, ABq,  $J=15\text{Hz}$ ), 4.4-5.1 (4H, m), 7.0-7.4 (14H, m), 7.58 (1H, br s), 7.75 (1H, m), 8.1 (3H, m), 8.48 (1H, d,  $J=8\text{Hz}$ ), 8.76 (1H, m), 9.3 (1H, br s)

(7)



mp :  $-205^{\circ}\text{C}$  (dec.)

IR (Nujol) : 3300, 1715, 1640, 1550  $\text{cm}^{-1}$

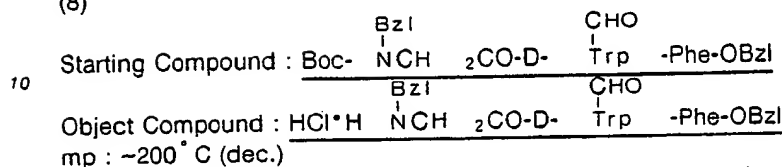
NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.82 (2H, m), 3.05 (2H, s), 3.17 (2H, s), 3.30 (2H, s), 4.4-4.9 (2H, m), 5.16 (2H, s), 7.26 (5H, s), 7.37 (5H, s), 7.2-7.5 (4H, m), 7.65 (1H, m), 8.2 (1H, br), 8.32 (1H, d,  $J=8\text{Hz}$ ), 8.87 (1H, d,  $J=8\text{Hz}$ ), 9.25 (1H, br s)

Elemental Analysis.

	Calculated for $C_{32}H_{32}N_4O_7$ :		
Found :	C 65.74, C 64.21,	H 5.52, H 5.35,	N 9.58 N 9.17

5

(8)



IR (Nujol) : 3270, 2600-2700 1710 (sh), 1680 (sh), 1695  $\text{cm}^{-1}$

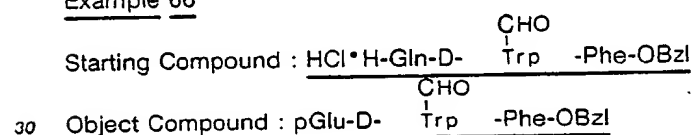
15 NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.78 (2H, m), 3.03 (2H, m), 3.58 (2H, s), 4.02 (2H, s), 4.4-4.9 (2H, m), 5.13 (28H, s), 7.25 (5H, s), 7.36 (5H, s), 7.44 (5H, s), 7.2-7.7 (4H, m), 8.15 (1H, br), 8.81 (1H, d,  $J=8\text{Hz}$ ), 8.96 (1H, d,  $J=8\text{Hz}$ ), 9.4 (2H, br)

Elemental Analysis.

20

	Calculated for $C_{37}H_{37}N_4O_5\text{Cl}$ :			
Found :	C 68.04, C 65.21,	H 5.71, H 5.47,	N 8.58, N 7.94,	Cl 5.43 Cl 2.47

25

Example 66

A mixture of  $\text{HCl}\cdot\text{H-Gln-D-Trp(CHO)-Phe-OBzl}$  (0.48 g) in AcOH (25 ml) was stirred for 8 hours at  $50^\circ\text{C}$ . After evaporation, the residue was pulverized with water. The white solid was filtered and washed successively with 2% hydrochloric acid, water, 2% sodium hydrogencarbonate and water, and dried. The

35 obtained powder was dissolved in DMF and reprecipitated with ethyl acetate. The precipitate was filtered and dried to give  $\text{pGlu-D-Trp(CHO)-Phe-OBzl}$  (0.35 g).

mp :  $205-209^\circ\text{C}$

IR (Nujol) : 3300, 1710, 1640, 1550  $\text{cm}^{-1}$

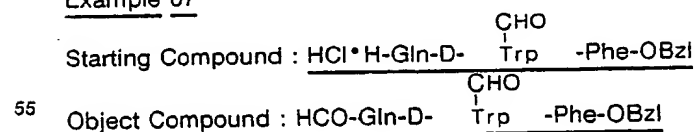
40 NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.3-1.8 (1H, m), 1.8-2.3 (3H, m), 2.6-3.3 (4H, m), 3.9-4.1 (1H, m), 4.4-4.9 (2H, m), 5.10 (2H, s), 7.1-7.5 (3H, m), 7.17 (5H, s), 7.29 (5H, s), 7.5-7.8 (2H, m), 8.08 (2H, br d,  $J=9\text{Hz}$ ), 8.72 (1H, d,  $J=8\text{Hz}$ ), 9.3 (1H, broad)

Elemental Analysis.

45

	Calculated for $C_{33}H_{32}N_4O_6$ :		
Found :	C 68.26, C 67.96,	H 5.55, H 5.57,	N 9.65 N 9.61

50

Example 67

To a solution of  $\text{HCl}\cdot\text{H-Gln-D-Trp(CHO)-Phe-OBzl}$  (0.33 g) and sodium formate (0.35 g) in formic acid (21 ml) was added dropwise  $\text{Ac}_2\text{O}$  (7 ml) under ice-cooling. The mixture was stirred for three and half an

hour at room temperature. Water (10 ml) was added to the mixture and then evaporated. To the residue, water was added and evaporated. The residue was pulverised with water, filtered. The solids were dissolved in DMF and reprecipitated with ethyl acetate, filtered and dried to give HCO-Gln-D-Trp(CHO)-Phe-OBzl (0.27 g).

5 mp :  $\sim 217^{\circ}\text{C}$  (dec.)

IR (Nujol) : 3300, 1710, 1660, 1640, 1550  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.3-2.2 (4H, m), 2.6-3.2 (4H, m), 4.1-4.9 (3H, m), 5.14 (2H, s), 6.7 (1H, br s), 7.0-7.8 (5H, m), 7.21 (5H, s), 7.32 (5H, s), 7.9-8.5 (4H, m), 8.73 (1H, br d,  $J = 8\text{Hz}$ ), 9.3 (1H, broad)

10

#### Example 68

Starting Compound : Boc-  $\begin{array}{c} \text{OTce} \\ | \\ \text{Glu} \end{array}$  -D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OBzl

15 Object Compound : Boc-Glu-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OBzl

To a solution of Boc-Glu(OTce)-D-Trp(CHO)-Phe-OBzl (0.40 g) in 90% AcOH (10 ml), was added zinc (0.20 g). The mixture was stirred for four and half an hour at room temperature. After filtration and evaporation, the residue was extracted with ethyl acetate. The organic layer was washed with water and saturated sodium chloride, and dried over magnesium sulfate. The evaporated residue was subjected to column chromatography on silica gel (20 g) and eluted with a mixture of chloroform and methanol (30:1 to 9:1, gradient elution). The fractions containing the object compound were combined and evaporated. The residue was pulverized with n-hexane, filtered and dried to give Boc-Glu-D-Trp(CHO)-Phe-OBzl (0.27 g).

mp :  $172-175^{\circ}\text{C}$

25 IR (Nujol) : 3320, 1720, 1710, 1690, 1640, 1545, 1525  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.32 (9H, s), 1.5-2.3 (4H, m), 2.6-3.4 (5H, m), 3.8-4.2 (1H, m), 4.4-4.9 (2H, m), 5.12 (2H, s), 6.7-7.0 (1H, m), 7.1-7.8 (4H, m), 7.25 (5H, s), 7.35 (5H, s), 7.9-8.4 (2H, m), 8.6-8.9 (1H, m), 9.3 (1H, broad)

Elemental Analysis.

30

	Calculated for $\text{C}_{38}\text{H}_{42}\text{N}_4\text{O}_9 \cdot 1/2\text{H}_2\text{O}$ :		
Found :	C 64.49, C 64.48,	H 6.12, H 5.98,	N 7.92 N 7.87

35

#### Example 69

Starting Compound : Boc-Gln-D-  $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$  -Phe-OBzl

Object Compound : Boc-Gln-D-Trp-Phe-OH

A mixture of Boc-Gln-D-Trp(CHO)-Phe-OBzl (1.2 g) and 1N sodium hydroxide (3.6 ml) in a mixture of THF (30 ml), methanol (10 ml) and water (5 ml) was stirred for 1.5 hours. After adding water (10 ml), the organic solvent was evaporated. The resulting aqueous solution was washed with diethyl ether, acidified with 10% citric acid solution and allowed to stand in a refrigerator overnight. The precipitates were filtered, washed with water and recrystallized with a mixture of ethanol and water to give Boc-Gln-D-Trp-Phe-OH (0.80 g).

50 mp :  $168-170^{\circ}\text{C}$

IR (Nujol) : 3320, 1715, 1690, 1645, 1545, 1530  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 1.4-2.2 (4H, m), 2.6-3.5 (4H, m), 3.7-4.1 (1H, m), 4.3-4.8 (2H, m), 6.6-7.6 (13H, m), 7.86 (1H, d,  $J = 8\text{Hz}$ ), 8.36 (1H, d,  $J = 9\text{Hz}$ ), 10.70 (1H, s), 12.7 (1H, broad)

Elemental Analysis.

55

	Calculated for $C_{30}H_{37}N_5O_7 \cdot 1/2H_2O$ :		
Found :	C 61.21, C 61.42,	H 6.51, H 6.31,	N 11.90 N 11.90

### Example 70

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 71.

Starting Compound : Boc-Gln-D- $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$ -Phe-OBzl

Object Compound : Boc-Gln-D-Trp-Phe-NH<sub>2</sub>

mp : 210-212 °C

IR (Nujol) : 3420, 3300, 3220, 1690, 1640, 1540, 1525 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.33 (9H, s), 1.4-2.1 (4H, m), 2.6-3.2 (4H, m), 3.7-4.1 (1H, m), 4.3-4.7 (2H, m), 6.6-7.6 (10H, m), 7.22 (5H, s), 7.7-8.0 (1H, m), 8.1-8.4 (1H, m), 10.73 (1H, s)

Elemental Analysis.

	Calculated for $C_{30}H_{38}N_5O_6$ :		
Found :	C 62.27, C 62.03,	H 6.62, H 6.59,	N 14.52 N 14.36

### Example 71

Starting Compound : Boc-D-Trp-Phe-OBzl

Object Compound : Boc-D-Trp-Phe-NH<sub>2</sub>

A mixture of Boc-D-Trp-Phe-OBzl (1.0 g) and 24% methanolic ammonia (20 ml) was allowed to stand at room temperature in a sealed tube for 18 hours. After evaporation, the residual crystals were collected and recrystallized from a mixture of water and ethanol to give Boc-D-Trp-Phe-NH<sub>2</sub> (0.63 g).

mp : 204-206 °C

IR (Nujol) : 3430, 3350, 1675, 1640, 1550, 1535 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 1.30 (9H, s), 2.5-3.4 (4H, m), 3.9-4.6 (2H, m), 6.68 (1H, br d, J=8Hz), 6.8-7.6 (12H, m), 8.13 (1H, br d, J=9Hz), 10.63 (1H, s)

Elemental Analysis.

	Calculated for $C_{25}H_{30}N_4O_4$ :		
Found :	C 66.65, C 66.92,	H 6.71, H 6.72,	N 12.44 N 12.33

### Example 72

The following object compound was obtained from the corresponding starting compound according to similar manners to those of Example 4 and Example 13, successively.

Starting compound: Boc-D- $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$ -Phe-OBzl

Object Compound: Z-D-Trp-D- $\overset{\text{CHO}}{\underset{|}{\text{Trp}}}$ -Phe-OBzl

mp : 169-173 °C

IR (Nujol) : 3300, 1710, 1690, 1645, 1540 cm<sup>-1</sup>NMR (DMSO-d<sub>6</sub>, δ) : 2.6-3.3 (6H, m), 4.1-5.0 (3H, m), 4.94 (2H, s), 5.13 (2H, s), 6.7-7.8 (25H, m), 8.0-8.4 (2H, m), 8.74 (1H, d, J = 8Hz), 9.2 (1H, broad)

5 Elemental Analysis.

	Calculated for C <sub>47</sub> H <sub>43</sub> N <sub>5</sub> O <sub>7</sub> :		
	C 71.47,	H 5.49,	N 8.87
Found :	C 71.61,	H 5.37,	N 8.87

10

15 Example 73

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 69.

Starting Compound: Z-D-Trp-D-  
 $\begin{array}{c} \text{CHO} \\ | \\ \text{Trp} \end{array}$ -Phe-OBzl

20 Object Compound: Z-D-Trp-D-Trp-Phe-OH

mp : 153-160 °C (dec.)

IR (Nujol) : 3600, 3400, 3300, 1740, 1670, 1640, 1565, 1540 cm<sup>-1</sup>NMR (DMSO-d<sub>6</sub>, δ) : 2.6-3.2 (6H, m), 3.2-3.6 (3H, broad), 4.1-4.9 (3H, m), 4.93 (2H, s), 6.8-7.5 (19H, m) 7.5-7.7 (2H, m), 7.9-8.2 (1H, m), 8.43 (1H, d, J = 9Hz), 10.74 (2H, s)

25 Elemental Analysis.

	Calculated for C <sub>39</sub> H <sub>37</sub> N <sub>5</sub> O <sub>6</sub> · H <sub>2</sub> O :		
	C 67.91,	H 5.70,	N 10.15
Found :	C 67.99,	H 5.58,	N 10.16

30

35 Example 74

The following object compound obtained from the corresponding starting compound according to similar manners to those of Example 15 and continuously Example 17.

Starting Compound :  $\begin{array}{c} \text{Tos} \\ | \\ \text{Boc-Thr-D-Trp-Phe-N} \end{array} \begin{array}{l} \text{Me} \\ \diagdown \\ \text{Bzl} \end{array}$

Object Compound :  $\begin{array}{c} \text{Tos} \\ | \\ \text{Ac-Thr-D-Trp-Phe-N} \end{array} \begin{array}{l} \text{Me} \\ \diagdown \\ \text{Bzl} \end{array}$

40

45

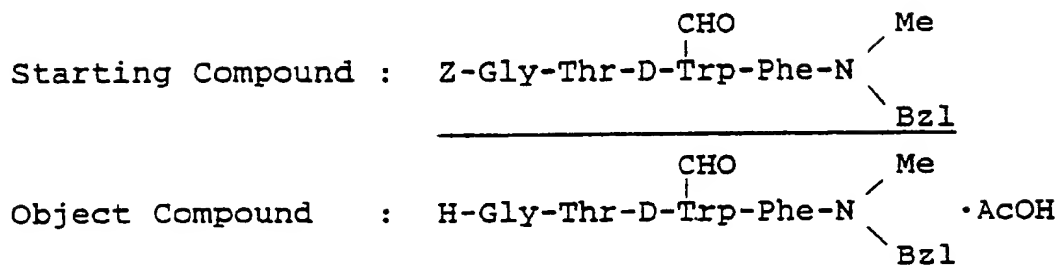
50 mp : 112-116 °C

IR (Numol) : 3400, 3250, 1660 (sh), 1640, 1170 cm<sup>-1</sup>NMR (DMSO-d<sub>6</sub>, δ) : 0.78 (3H, d, J = 6Hz), 1.97 (3H, s), 2.27 (3H, s), 2.80 (3H, s), 2.6-3.1 (4H, m), 3.75 (1H, m), 4.1 (1H, m), 4.3-5.0 (5H, m), 6.9-7.35 (14H, m), 7.5-7.9 (6H, m), 8.05 (1H, d, J = 6Hz), 7.60 (1H, t, J = 6Hz)

55 Elemental Analysis.



	Calculated for C <sub>41</sub> H <sub>45</sub> N <sub>5</sub> O <sub>7</sub> S :		
Found :	C 65.49, C 64.80,	H 6.03, H 6.03,	N 9.31 N 9.24

Example 75

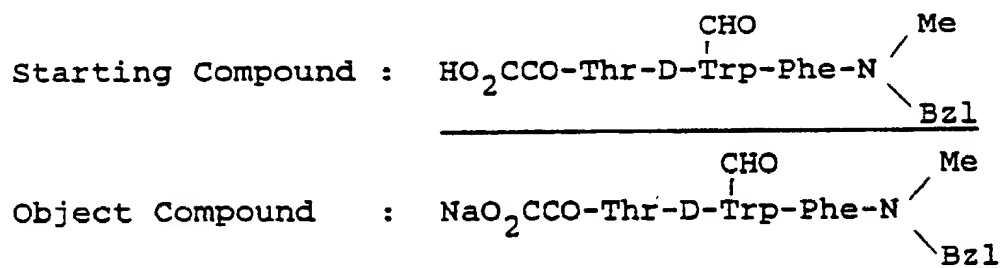
A solution of Z-Gly-Thr-D-Trp(CHO)-Phe-NMeBzl(560 mg) in a mixed solvent of ethanol (30 ml) and acetic acid (10 ml) was hydrogenated over 10% palladium on carbon (350 mg) under atmospheric pressure for two hours. After filtration of the catalyst and evaporation, the residue was dissolved in water (50 ml) and lyophilized to give H-Gly-Thr-D-Trp(CHO)-Phe-NMeBzl·AcOH (230 mg).

IR (Nujol) : 3300, 1720 (sh), 1690 (sh), 1660 (sh), 1640 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>/D<sub>2</sub>O, δ) : 0.80 (3H, d, J=6Hz), 2.80 and 2.97 (3H,s), 2.6-3.0 (4H, m), 3.27 (2H, m), 4.3-5.1 (5H, m), 7.20 (5H, s), 6.8-7.6 (10H, m), 8.0 (1H, br), 9.1 (1H, br)

Example 76

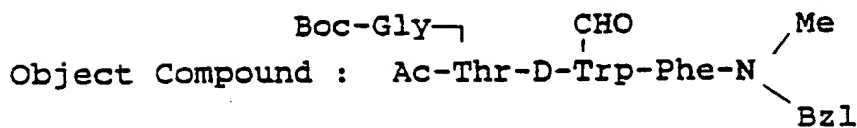
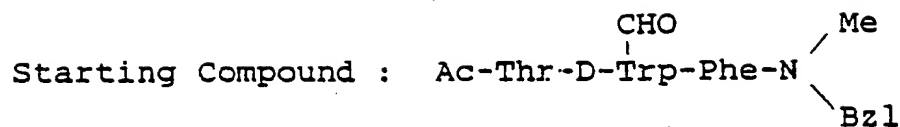
The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 79.



IR (Nujol) : 3300, 1710, 1685, 1660, 1640 cm<sup>-1</sup>

NMR (D<sub>2</sub>O, δ) : 1.03 (3H, d, J=6Hz), 2.37 and 2.63 (3H, s), 2.5 (2H, m), 2.9 (2H, m), 3.7 (1H, m), 4.0-4.3 (2H, m), 5.4 (1H, m) 6.6-7.4 (14H, m), 8.9 (1H, m), 9.8 (1H, br s)

Example 77

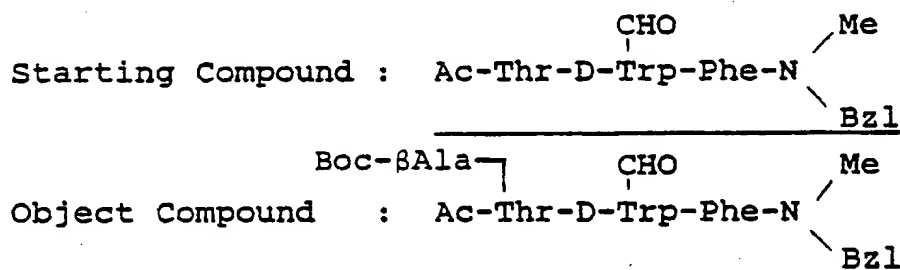


To a solution of Ac-Thr-D-Trp(CHO)-Phe-NMeBzl (1.07 g), Boc-Gly-OH (0.39 g) and 4-dimethylaminopyridine (125.3 g) in DMF (16 ml) was added WAC·HCl (392 mg) at room temperature. After stirring the solution overnight, Boc-Gly-OH (175 mg) and WSC·HCl (191 mg) were added thereto, and the solution was further stirred for 18 hours. The solution was concentrated under vacuum, and the product was extracted with ethyl acetate. The extract was washed successively with water, diluted sodium hydrogencarbonate solution, 0.5N hydrochloric acid, and sodium chloride solution and dried over magnesium sulfate. The crude product was purified on a silica gel column chromatography (25 g) eluting with chloroform-methanol (100:2 to 100:2.5) to give Ac-Thr(Boc-Gly)-D-Trp(CHO)-Phe-NMeBzl (1.26 g) as an amorphous solid.

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d, J=6Hz), 1.37 (9H, s), 1.83 (3H, s), 2.83 (3H, s), 2.7-3.1 (4H, m), 3.55 (2H, d, J=6Hz), 4.28 and 4.63 (2H, ABq, J=15Hz), 4.4-5.1 (4H, m), 6.9-7.5 (14H, m), 7.77 (1H, m), 8.0 (1H, t, J=7Hz), 8.15 (1H, m), 8.30 (1H, d, J=7Hz), 8.67 (1H, m), 9.30 (1H, br s)

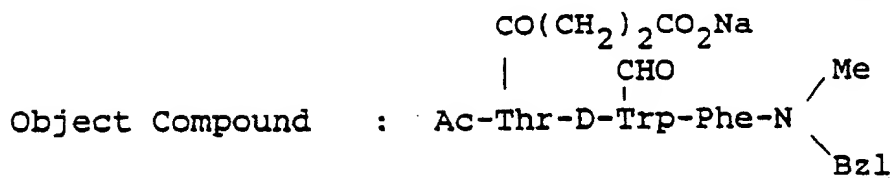
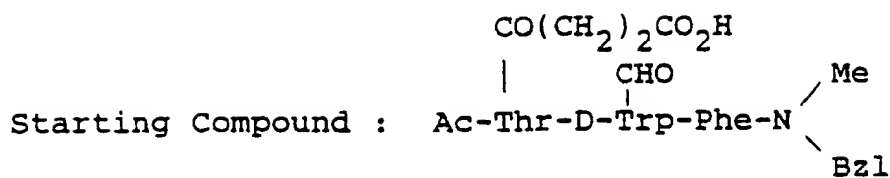
#### Example 78

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 77.



NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.83 (3H, d, J=7Hz), 1.37 (9H, s), 1.87 (3H, s), 2.24 (2H, t, J=7Hz), 2.87 (3H, s), 2.6-3.0 (4H, m), 3.05 (2H, m), 4.30 and 4.68 (2H, ABq, J=15Hz), 4.4-5.1 (4H, m), 6.67 (1H, m), 6.95-7.55 (14H, m), 7.6 (1H, m), 7.90 (1H, d, J=8Hz), 8.1 (1H, m), 8.34 (1H, d, J=8Hz), 8.70 (1H, m), 9.25 (1H, br s)

#### Example 79



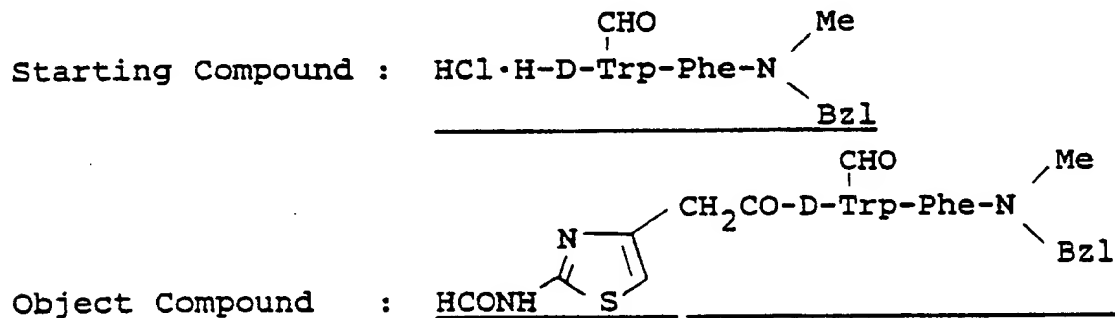
Ac-Thr(CO(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>H)-D-Trp(CHO)-Phe-NMeBzl (482 mg) was dissolved in acetone (10 ml) and sodium 2-ethyl-hexanoate (111 mg) at room temperature. The mixture was stirred for 20 minutes at the same temperature, and the precipitates were collected, washed with acetone, and dried under vacuum to give Ac-Thr(CO(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>Na)-D-Trp(CHO)-Ohe-NMeBzl (300 mg).

IR (Numol) : 3250, 1740 (sh), 1710, 1640, 1590 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.80 (3H, d, J=6Hz), 1.85 (3H, s), 2.25 (4H, s), 2.78 and 2.81 (3H, s), 2.85-3.1 (4H, m), 4.2-5.0 (6H, m), 6.95-7.4 (13H, m), 7.6 (2H, m), 8.1 (2H, m), 8.9 (1H, d, J=7Hz), 9.2 (1H, m)

#### Example 80

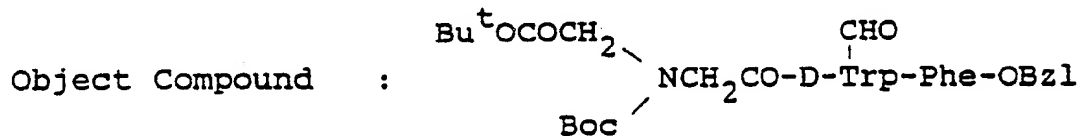
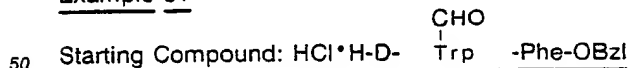
The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 64.



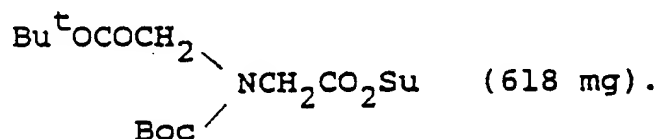
IR (Nujol) : 3270, 3180, 1705, 1790, 1660, 1630 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 2.80 (2H, s), 2.88 (3H, s), 2.7-2.9 (2H, m), 3.47 (2H, s), 4.33 and 4.63 (2H, ABq, J=15Hz), 4.65 (1H, m), 5.04 (1H, m), 6.73 (1H, s), 7.0-7.5 (14H, m), 7.67 (1H, m), 8.20 (1H, d, J=8Hz), 8.45 (1H, s), 8.78 (1H, m), 9.25 (1H, br), 12.1 (1H, br)

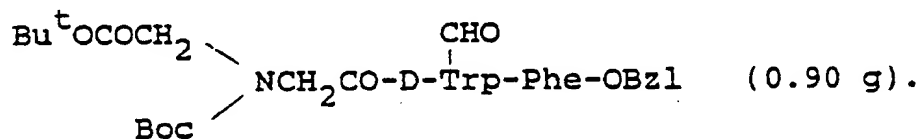
#### Example 81



To an ice-cooled solution of HCl·H-D-Trp(CHO)-Phe-OBzl (800 mg) and NMM (162 mg) in DMF (15 ml) was added



The solution was stirred for two hours under ice-cooling and for two and half hours at room temperature, and to the reaction mixture were added NMM (72 mg) and the active ester (50 mg). After stirring for additional three hours, N,N-dimethyl-1,3-propanediamine (3 drops) was added and the mixture was stirred further for an hour. After concentration, the product was extracted with ethyl acetate and the extract was washed successively with water, diluted sodium hydrogencarbonate solution, 0.5N hydrochloric acid, and sodium chloride solution, and dried over magnesium sulfate. The crude product was purified on a silica gel column (30 g) elution with chloroform-methanol (100:1) to give a purified product which was crystallized with diisopropyl ether to give



mp : 126-127° C

IR (Nujol) : 3300, 1740, 1710, 1690, 1670, 1650 cm<sup>-1</sup>

NMR (CDCl<sub>3</sub>, δ) : 1.30, 1.36 and 1.46 (18H, s), 3.0-3.4 (4H, m), 3.6-4.2 (4H, m), 4.7-5.0 (2H, m), 5.10 (2H, s), 7.9 (1H, m), 7.1-7.5 (14H, m) 7.6 (1H, m) 8.4 (1H, d, J = 7Hz), 9.1 (1H, br s)

#### Example 82

The following compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 58.

(1)

Starting Compound : Z-D-Trp-Phe-OBzl

Object Compound : H-D-Trp-Phe-OH

mp : -249° C (dec.)

IR (Nujol) : 3250, 1690, 1605, 1535 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 2.6-3.3 (4H, m), 3.7-4.0 (1H, m), 4.2-4.6 (1H, m), 6.53 (3H, br s), 6.9-7.3 (8H, m), 7.23-7.5 (1H, m), 7.5-7.8 (1H, m), 8.3 (1H, broad), 10.95 (1H, s)

Elemental Analysis.

	Calculated for C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> :		
Found :	C 68.36, C 68.25,	H 6.02, H 5.93,	N 11.96 N 12.01

(2)

Starting Compound : Boc-D-Trp-Phe-OBzl

Object Compound: Boc-D-Trp-Phe-OH

mp : 190-200° C

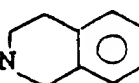
IR (Nujol) : 3400, 3300, 1720, 1680, 1650, 1525 cm<sup>-1</sup>NMR (DMSO-d<sub>6</sub>, δ) : 1.29 (9H, s), 2.5-3.2 (4H, m), 3.27 (4H, broad, overlapped with H<sub>2</sub>O), 4.0-4.6 (2H, m), 6.51 (1H, br d, J=8Hz), 6.8-7.0 (3H, m), 7.0-7.6 (2H, m), 7.17 (5H, s), 8.11 (1H, br d, J=8Hz), 10.62 (1H, s)

5 Elemental Analysis.

	Calculated for C <sub>25</sub> H <sub>29</sub> N <sub>3</sub> O <sub>5</sub> :		
Found :	C 66.50, C 66.13,	H 6.47, H 6.39,	N 9.31 N 9.32

Example 83

15 The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 8.

20 Starting Compound : Boc-Phe-N 

25 Object Compound : 

30 mp : 216-218° C

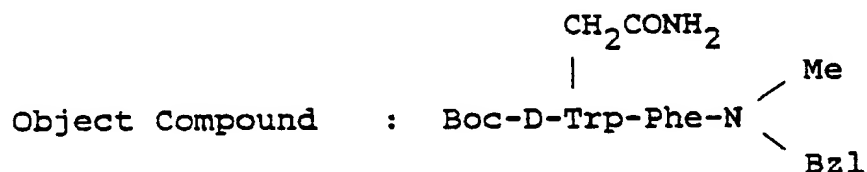
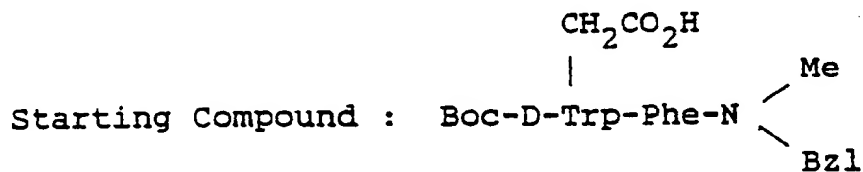
IR (Nujol) : 3360, 1720, 1705, 1705, 1655, 1630, 1515 cm<sup>-1</sup>NMR (DMSO-d<sub>6</sub>, δ) : 1.06 (s) and 1.26 (s) (9H), 2.5-3.1 (6H, m), 3.5-3.7 (1H, m), 3.7-3.9 (1H, m), 4.1-4.3 (1H, m), 4.4-4.8 (2H, m), 5.0-5.2 (1H, m), 6.8-7.0 (1H, m) 7.0-8.3 (14H, m), 8.5-8.8 (1H, m), 9.22 (s) and 9.61 (s) (1H)

35 Elemental Analysis.

	Calculated for C <sub>35</sub> H <sub>38</sub> N <sub>4</sub> O <sub>5</sub> :		
Found :	C 70.69, C 70.33,	H 6.44, H 6.46,	N 9.42 N 9.32

Example 84

45 The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Preparation 1-(1).



IR ( $\text{CH}_2\text{Cl}_2$ ) : 3490, 3400, 3350, 1710, 1670 (sh), 1640  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 1.29 (9H, s), 2.65-3.05 (4H, m), 2.78 and 2.88 (3H, s), 4.23 (1H, m), 4.41 and 4.57 (2H, ABq,  $J=14\text{Hz}$ ), 4.68 (2H, s), 4.9-5.1 (1H, m), 6.6-6.75 (1H, m), 6.907.4 (16H, m), 7.6-7.8 (1H, m), 8.5-8.67 (1H, m)

#### Example 85

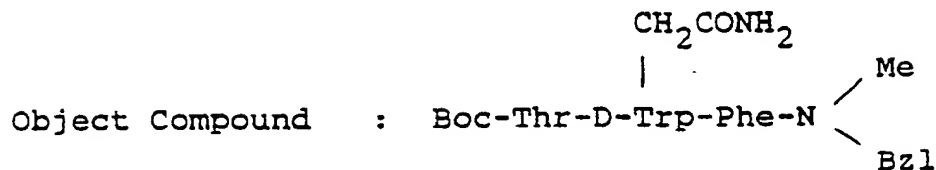
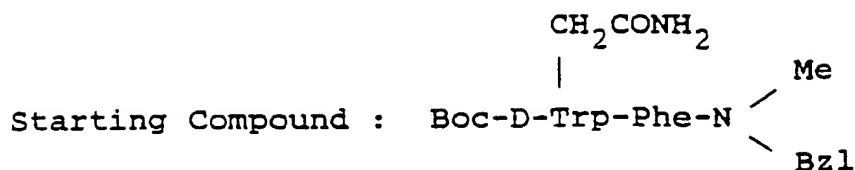
The following object compounds were obtained from the corresponding starting compounds according to similar manners to those of Example 4 and Example 13, successively.



IR (Nujol) : 3300, 1710, 1655-1625  $\text{cm}^{-1}$

NMR ( $\text{DMAO-d}_6$ ,  $\delta$ ) : 1.7-1.9 (3H, m), 1.34 (9H, s), 2.5-3.1 (6H, m), 3.4-3.6 (1H, m), 3.6-3.9 (3H, m), 4.4-4.8 (4H, m), 5.0-5.1 (1H, m), 6.32 (1H, d,  $J=8\text{Hz}$ ), 7.1-7.7 (13H, m), 7.9-8.3 (2H, m), 8.5-8.8 (1H, m), 9.13 (s) and 9.61 (s) (1H)

(2)



IR (Nujol) : 3300, 1710 (sh), 1690 (sh), 1680 (sh), 1630  $\text{cm}^{-1}$

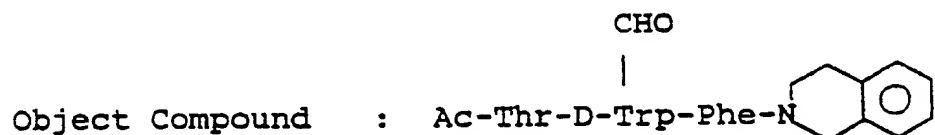
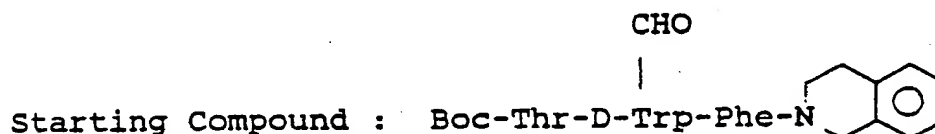
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.84 (3H, d,  $J = 5.6\text{Hz}$ ), 1.37 (9H, s), 2.76 and 2.84 (3H, s), 2.6-3.0 (4H, m), 3.7-3.95 (2H, m), 4.27-4.78 (6H, m), 4.85-5.0 (1H, m), 6.3 (1H, m), 6.95-7.4 (16H, m) 7.5-7.6 (1H, m), 7.9-8.0 (1H, m), 8.5-8.65 (1H, m)

Elemental Analysis.

	Calculated for $\text{C}_{39}\text{H}_{48}\text{N}_6\text{O}_7 \cdot \text{H}_2\text{O}$ :		
Found :	C 64.09,	H 6.90,	N 11.50
	C 64.17,	H 6.70,	N 11.35

### Example 86

The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 23.

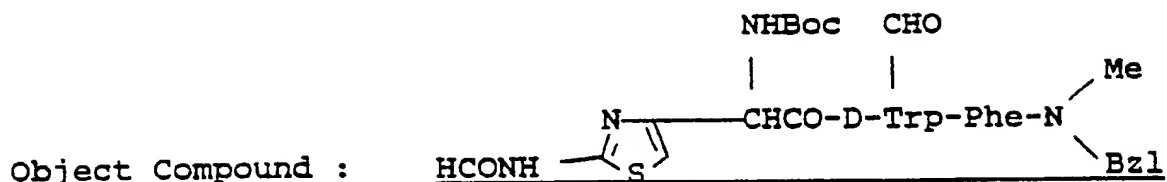
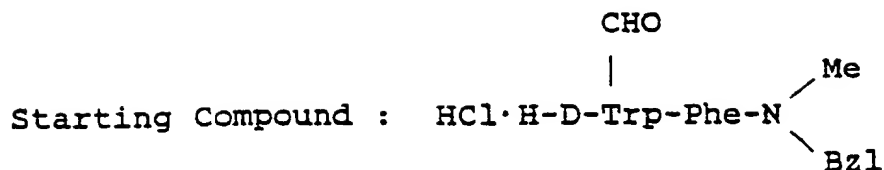


IR (Nujol) : 3270, 1705, 1640, 1545  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.74 (3H, d,  $J = 5\text{Hz}$ ), 1.84 (3H, s), 2.5-3.1 (6H, m), 3.4-3.6 (1H, m), 3.6-3.9 (2H, m), 4.0-4.1 (1H, m), 4.4-4.8 (4H, m), 4.95-5.1 (1H, m), 7.1-7.5 (12H, m), 7.5-7.8 (2H, m), 7.9-8.3 (2H, m), 8.6-8.8 (1H, m), 9.14 (s) and 9.60 (s) (1H)

### Example 87

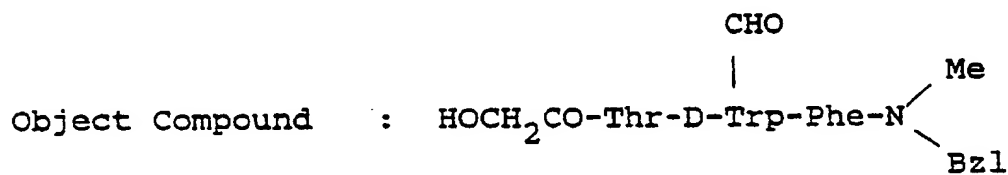
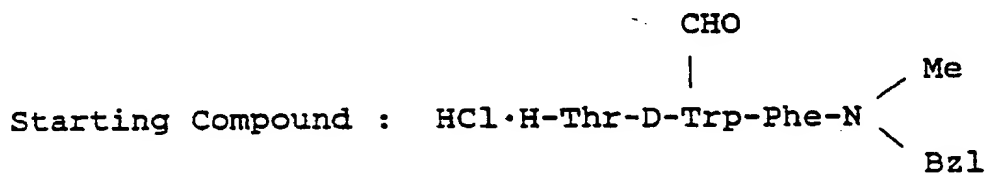
The following object compounds were obtained from the corresponding starting compounds according to a similar manner to that of Example 13.



IR (Nujol) : 3300, 1710, 1690, 1670, 1655 (sh), 1640, 1630, 1545  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.34 and 1.36 (9H, s), 2.7-3.1 (7H, m), 4.3-4.5 (1H, m), 4.6-4.8 (2H, m), 4.9-5.2 (1H, m), 5.24 (1H, d,  $J=8\text{Hz}$ ), 6.68 (1H, d,  $J=8\text{Hz}$ ), 7.0-7.4 (15H, m), 7.64 (1H, m), 8.22 (1H, m), 8.44 and 8.49 (1H, s), 8.7-9.2 (1H, m), 12.1-12.4 (1H, m)

(2)



IR (Nujol) : 3300, 1710, 1640, 1535  $\text{cm}^{-1}$

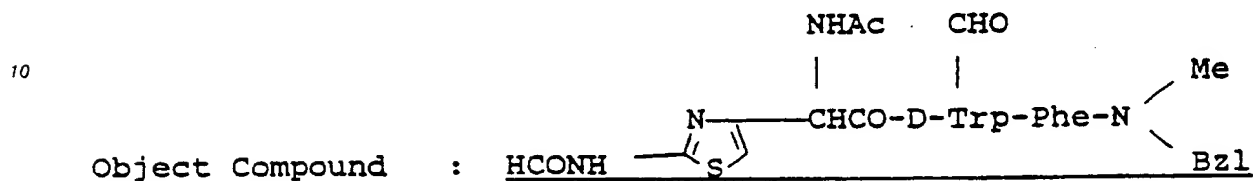
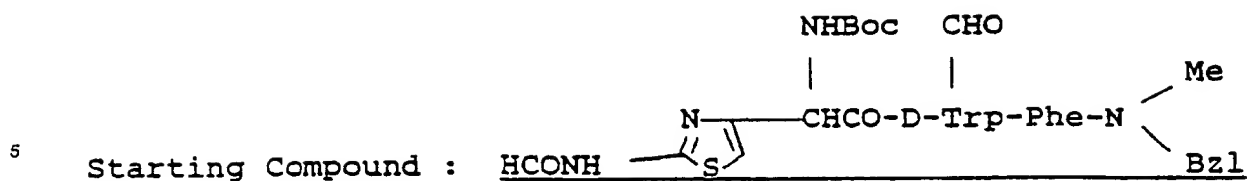
NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.71 (3H, br), 2.80 and 2.89 (3H, s), 2.6-3.1 (4H, m), 3.18 (1H, br), 3.86 (2H, s), 4.1-4.2 (1H, m), 4.5-4.8 (2H, m), 4.82-5.05 (2H, m), 5.7 (1H, br), 7.0-7.4 (13H, m), 7.4-7.6 (1H, m), 7.7 (1H, br), 7.9-8.3 (2H, m), 8.70 and 8.80 (1H, d,  $J=8\text{Hz}$ ), 9.15 and 9.60 (1H, s)

#### Example 88

The following object compounds were obtained from the corresponding starting compounds according to similar manners to those of Example 2 and Example 17, successively.

(1)





15 The product was a mixture of two enantiomers and used in the next reaction without separation.

This crude product was suspended in ethyl acetate and heated with water bath under reflux. After cooling to room temperature, the precipitates was collected, washed with ethyl acetate, and dried to give one of the enantiomers (HPLC RT=4.7 min, isomer A). The filtrate was applied to silica gel column and  
20 eluted with chloroformmethanol (100:3) to give another enantiomer (HPLC RT-5.1 min, isomer B) which was triturated with diisopropyl ether.

25 isomer A

mp : 218-220 °C

IR (Nujol) : 3280, 1690, 1670 (sh), 1645 (sh), 1632, 1535 cm<sup>-1</sup>

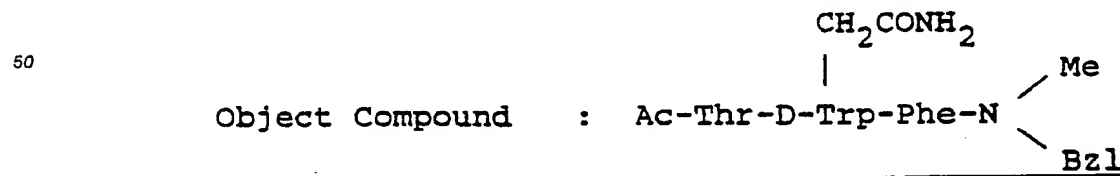
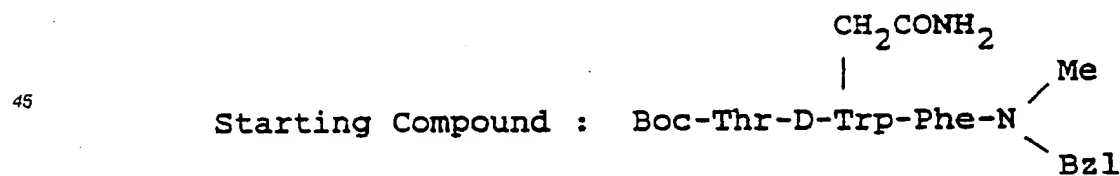
NMR (DMSO-d<sub>6</sub>, δ) : 1.845 (3H, s), 2.82 and 2.92 (3H, s), 2.6-3.1 (4H, m), 4.33-4.40 and 4.53-4.80 (3H, m),  
30 5.0 (1H, m), 5.50 (1H, d, J=8Hz), 6.5-6.75 (1H, m), 7.0-7.4 (13H, m), 7.68 (1H, br s), 7.9-8.4 (3H, m), 8.44 (1H, s), 8.79 and 8.88 (1H, d, J=8Hz), 9.05 and 9.58 (1H, br s), 12.21 (1H, s)

isomer B

IR (3288-16) : 3280, 1715-1610, 1550-1510 cm<sup>-1</sup>

35 NMR (DMSO-d<sub>6</sub>, δ) : 1.88 (3H, s), 2.81 and 2.89 (3H, s), 2.7-3.1 (4H, m), (3288-15) 4.3-8 (3H, m), 4.9-5.1 (1H, m), 5.56 (1H, d, J=8Hz), 7.0-7.4 (13H, m), 7.5-7.7 (2H, m), 8.0-8.8, 9.17 and 9.62 (5H, m), 12.41 and 12.80 (1H, m)

(2)



55 mp : 230-232 °C (dec.)

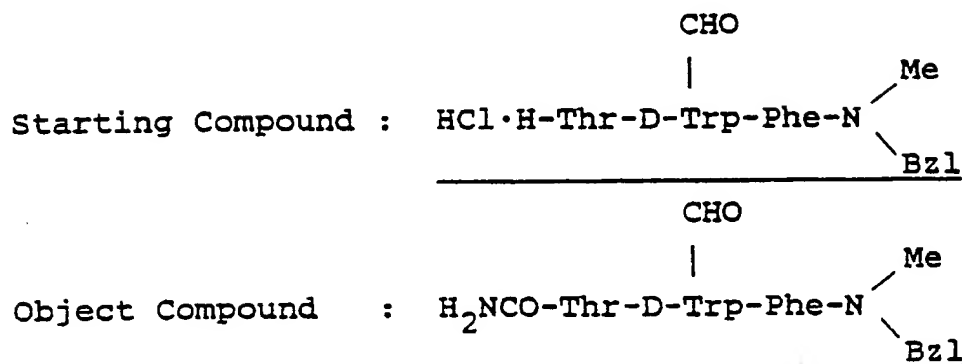
IR (Nujol) : 3390, 3290, 1680, 1670 (sh), 1634, 1530 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>, δ) : 0.8 (3H, m), 1.87 (3H, s), 2.77 and 2.85 (3H, s), 2.7-3.14 (4H, m), 3.8 (1H, m), 4.1 (1H,

m), 4.3-4.8 (6H, m), 4.85-5.0 (1H, m), 6.95-7.4 (17H, m), 7.6 (1H, m), 7.8-8.0 (2H, m), 8.5-8.7 (1H, m)  
Elemental Analysis.

	Calculated for $C_{30}H_{42}N_6O_6 \cdot H_2O$ :		
Found :	C 64.27, C 64.69,	H 6.59, H 6.60,	N 12.49 N 12.64

#### Example 89



To a solution of  $HCl \cdot H-Thr-D-Trp(CHO)-Phe-NMeBzl$  (0.94 g) and triethylamine (0.153 g) in acetonitrile (12 ml), was added chlorosulfonyl isocyanate (0.214 g) under cooling with Dry ice and carbon tetrachloride bath. The solution was stirred at the same temperature for an hour and then stirred under ice-cooling. Chlorosulfonyl isocyanate (0.214 g) was added at this temperature, after stirring for fifteen minutes, water (3 ml) was added. The pH was adjusted to pH 4 with sodium hydrogencarbonate and the mixture was stirred for an hour. After evaporation of acetonitrile, the product was extracted with ethyl acetate under saturation with sodium chloride. The organic layer was washed with sodium chloride solution and concentrated. The residue was dissolved in  $CH_3CN-H_2O$  (8:2) (20 ml) and applied to a column of  $\text{TOYO PEARL HW-40}$  (26 mm $\phi$ , 400 ml) and eluted with  $CH_3CH-H_2O$  (7:3), and fractionated. The main fraction was collected, and after evaporation of acetonitrile, n-butanol and ethyl acetate was added and the organic layer was separated and concentrated to give  $H_2NCO-Thr-D-Trp(CHO)-Phe-NMeBzl$  (600 mg).

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.6-0.8 (3H, m), 2.83 and 2.92 (3H, s), 2.7-3.1 (4H, m), 3.84 (1H, d,  $J = 5\text{Hz}$ ), 4.1-4.4 and 4.5-5.1 (4H, m) 7.0-7.4 (15H, m), 7.5-7.9 (1H, m) 8.2-8.6 (3H, m), 8.9-9.6 (1H, m)

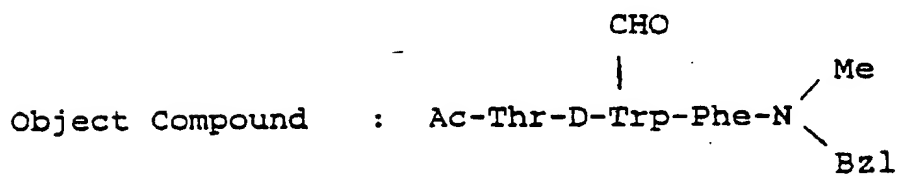
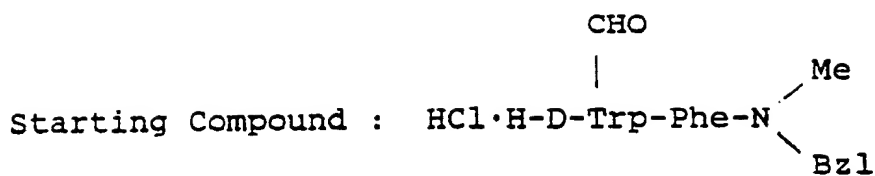
Column : Lichrosob RP-18 (4 x 250nm),

Eluant : MeOH- $H_2O$  (75:25) 0.1% trifluoroacetic acid,

Flow rate : 1.5 ml/min, Detection : UV 254 nm

#### Example 90

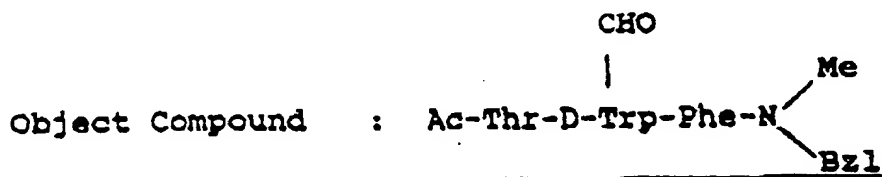
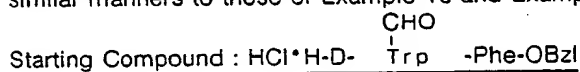
The following object compound was obtained from the corresponding starting compound according to a similar manner to that of Example 13.



IR (Nujol) : 3450 (sh), 3260, 1720 (sh), 1698, 1660 (sh), 1645-1620 (broad), 1550  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 1.87 (3H, s), 2.80 (s) and 2.87 (s) (3H), 2.6-3.2 (4H, m), 3.6-3.9 (1H, m), 3.95-4.3 (1H, m), 4.3-5.2 (5H, m), 6.95-7.8 (15H, m), 7.8-8.3 (2H, m), 8.5-8.75 (1H, m), 9.0-9.7 (1H, br s)

#### Example 91.

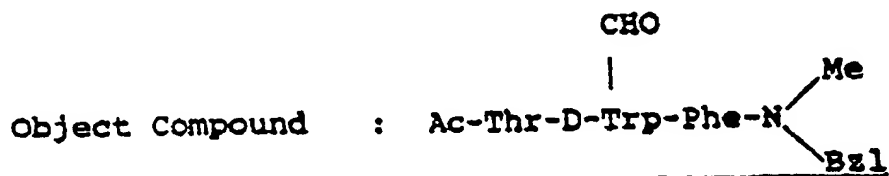
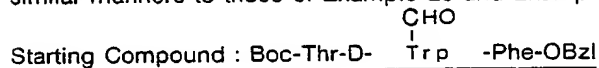
The following object compound was obtained from the corresponding starting compound according to similar manners to those of Example 13 and Example 71, successively,



IR (Nujol) : 3450 (sh), 3260, 1720 (sh), 1698, 1660 (sh), 1645-1620 (broad), 1550  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 1.87 (3H, s), 2.80 (s) and 2.87 (s) (3H), 2.6-3.2 (4H, m), 3.6-3.9 (1H, m), 3.95-4.3 (1H, m), 4.3-5.2 (5H, m), 6.95-7.8 (15H, m), 7.8-8.3 (2H, m), 8.5-8.75 (1H, m), 9.0-9.7 (1H, br s)

#### Example 92

The following object compound was obtained from the corresponding starting compound according to similar manners to those of Example 23 and Example 71, successively.



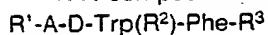
IR (Nujol) : 3450 (sh), 3260, 1720 (sh), 1698, 1660 (sh), 1645-1620 (broad), 1550  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.80 (3H, d,  $J=6\text{Hz}$ ), 1.87 (3H, s), 2.80 (s) and 2.87 (s) (3H), 2.6-3.2 (4H, m), 3.6-3.9 (1H, m), 3.95-4.3 (1H, m), 4.3-5.2 (5H, m), 6.95-7.8 (15H, m), 7.8-8.3 (2H, m), 8.5-8.75 (1H, m), 9.0-9.7 (1H, br s)

br s)

## Claims

5

1. A compound of the formula :

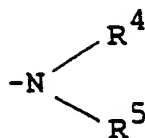


wherein

R<sup>1</sup> is hydrogen or an amino protective group,10 R<sup>2</sup> is hydrogen, an amino protective group, carbamoyl(lower) alkyl, carboxy(lower) alkyl or protected carboxy(lower) alkyl,R<sup>3</sup> is ar(lower) alkyl,

a group of the formula:

15



20

wherein R<sup>4</sup> and R<sup>5</sup> are each hydrogen, aryl or lower alkyl which may hve suitable substituent(s),  
orR<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-condensed lower alkylene, or

25 a group of the formula :

wherein R<sup>6</sup> is hydrogen, aryl or lower alkyl which may have suitable substituent(s), andA is a single bond or one or two amino acid(s) residue, provided that when A is one amino acid residue of  
-D-Trp-, then R<sup>4</sup> is not hydrogen,

30 and a pharmaceutically acceptable salt thereof.

2. A compound of the formula :

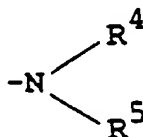


wherein

R<sup>1</sup> is hydrogen or an amino protective group,35 R<sup>2</sup> is hydrogen, an amino protective group, carbamoyl(lower)alkyl, carboxy(lower)alkyl or protected carboxy-(lower)alkyl,R<sup>3</sup> is ar(lower)alkyl,

a group of the formula:

40



45

wherein R<sup>4</sup> is hydrogen, aryl or lower alkyl which may have suitable substituent(s), andR<sup>5</sup> is aryl or lower alkyl which may have suitable substituent(s), orR<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-condensed lower alkylene, or

50 a group of the formula:

wherein R<sup>6</sup> is aryl or lower alkyl which may have suitable substituent(s). and

A is a single bond or one or two amino acid(s) residue,

and a pharmaceutically acceptable salt thereof.

55

3. A compound of claim 2, wherein

A is one or two amino acid(s) residue.

- 19 -

as agonists or antagonists with the neurokynin A (NKA) receptor has been valued in a in vitro test using the pulmonary artery of a rabbit (RPA) (Rovero et al., Neuropeptides, 1989, 13, 263-270) and their activity was determined as  $pK_B$  (antilogarithm of the dissociation constant), as described in Jenkinson et al., *TIPS*, 12, 53-56, 1991. For example, compound 2 has shown a  $pK_B = 8.67$ . The capability of the products of the present invention to interact as agonists or antagonists with NKA receptor has been valued in vivo as capability, after intravenous administration, to inhibit the agonist [betaAla<sup>8</sup>] NKA (4-10)-induced contractions of the urinary bladder in the anaesthetized mouse, as described in Maggi et al., *J. Pharmacol. Exp. Ther.*, 1991, 257, 1172. Compound 1, e.g., causes, at dose of 10 nmol/Kg i.v., an inhibitory effect of 50-70 %, as it has been valued at different times. The effect lasts over a period of more than 3 hours.

## ABBREVIATIONS:

Asn( $\beta$ -D-Glc):  $N^G$ -( $\beta$ -D-glucopiranosyl)-L-asparagine

Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]:  $N^G$ -(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopiranosyl)-L-asparagine

20 Fmoc-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp:  $N^G$ -(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopiranosyl) $N^a$ -(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl esthere

Ser( $\beta$ -D-Glc):  $O^G$ -( $\beta$ -D-glucopiranosyl)-L-asparagine

Ser[(Bz<sub>4</sub>O)- $\beta$ -D-Glc]:  $O^G$ -(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-glucopiranosyl)-L-asparagine

25 Fmoc-Ser[(Bz<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp:  $O^G$ -(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-

- 20 -

glucopyranosyl)N<sup>a</sup>-(fluoren-9-ylmethoxycarbonyl)-L-serine  
pentafluorophenyl ester.

Glc: glucopyranosyl

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

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- (G) TELEPHONE: 055-56801
- (H) TELEFAX: 055-5680615

(ii) TITLE OF INVENTION: Bicyclic compounds, preparation thereof  
and use in pharmaceutical compositions

(iii) NUMBER OF SEQUENCES: 35

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: IT FI 95 A 000044
- (B) FILING DATE: 13-MAR-1995

## (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

## (2) INFORMATION FOR SEQ ID NO: 1:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-Glc), wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Trp Phe Xaa Leu  
1                    5

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-Glc), wherein Glc is glucopyranosyl



## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-amino-Glc), wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) **FEATURE:**

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Xaa is Nle, i.e. norleucine

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 6  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-acetamido  
-Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: N1e and Asn are bound together to form a first cycle

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Xaa Asp Trp Phe Xaa Leu  
1 5

1

5

(2) INFORMATION FOR SEQ ID NO: 6:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-ribofuranosyl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-ribofuranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -L-arabinofuranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -L-arabinofuranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ser Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-mannopyranosil)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-mannopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-galactopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-galactopyranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-glucuronopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Asn Asp Trp Phe Xaa Leu  
1                    5

## (2) INFORMATION FOR SEQ ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-glucuronopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(1-deoxy-sorbitol-1-yl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Asp Trp Phe Xaa Leu  
1                      5

## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc], wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn[4-O-( $\beta$ -D-galactopyranosyl  
- $\beta$ -D-Glc]

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

( 1x ) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn[O- $\alpha$ -D-Glc-(1 $\rightarrow$ 4)-O- $\alpha$ -D-Glc-(1 $\rightarrow$ 4)- $\alpha$ -D-Glc], wherein Glc is glucopyranosyl

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cycle

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-glucopyranos-2-yl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Dap[D(-)-quinyl]

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap[D(-)-quinyl] and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 22:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap[D-gluconyl]

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap[D-gluconyl] and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap[D-glucuryl]

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap[D-glucuryl] and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Xaa is Dap(sulfo-benzoyl)

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Dap(sulfo-benzoyl) and Leu are bound together to form a first cyclo

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(4-sulfo-phenyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -L-Glc), wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-mannopyranos-2-yl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 29:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-galactopyranos  
2-yl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound  
together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to  
form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 30:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-xylopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(3-sulfo-propionyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 32:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap(Lysyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap(Lysyl) and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap(Arginyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap(Arginyl) and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 34:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap(4-O- $\beta$ -D-galactopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap(4-O- $\beta$ -D-galactopyranosyl) and Le

are bound together to form a first cycl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 35:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn(2-deoxy-2-trifluoro-acetoamido- $\beta$ -D-Glc, wherein Glc is glucopyranosyl

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

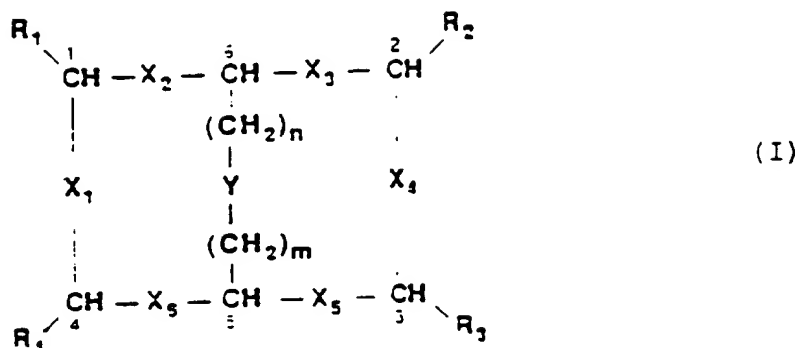
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Xaa	Asp	Trp	Phe	Xaa	Leu
1				5	

## CLAIMS

1. Bicycl compounds of general Formula



- wherein  $\text{X}_1$ ,  $\text{X}_2$ ,  $\text{X}_3$ ,  $\text{X}_4$ ,  $\text{X}_5$  and  $\text{X}_6$ , same or different from one another, represent a  $-\text{NR}'\text{CO}-$  or a  $-\text{CONR}'-$  group, where  $\text{R}'$  is H or  $\text{C}_{1-3}$  alkyl; Y represents a group selected from  $-\text{NRCO}-$ ,  $-\text{CONR}-$  or  $-\text{SS}-$  wherein R is H or  $\text{C}_{1-3}$  alkyl; at least one of  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$  groups, same or different from one another, is hydrophilic and the remaining groups are hydrophobic; m and n, same or different from one another, are each an integer number from 1 to 4.

2. Compounds as claimed in claim 1, wherein the hydrophobic groups can be separately selected from the following:

- groups corresponding to  $\text{C}_n\text{H}_{2n+1}$  wherein  $n = 0, 1-4$ ;
- linear or branched-alkyl groups corresponding to  $\text{C}_n\text{H}_{2n}-\text{U}-\text{W}$  wherein  $n = 1-4$ ;  $\text{U} = \text{O}$ ,  $\text{COO}$ ,  $\text{CONH}$ , S and  $\text{W} = \text{alkyl-}$ ,  $\text{aryl-}$  or  $\text{alkylaryl-group}$  containing from 1 to 15 C atoms;
- $(\text{CH}_2)_n-\text{C}_6\text{H}_3-\text{A}-\text{B}$  wherein  $n = 0, 1-3$ ; A and B, placed in any of the ortho, meta or para positions, same or different from one another, represent H, halogen, OR,  $\text{NHR}$ ,  $\text{NR}_2$ ,  $\text{CH}_3$ , SR wherein R is an alkyl-, aryl- or alkylaryl-group with less than 10 C atoms;

- 11 d)  $(\text{CH}_2)_n\text{-C}_6\text{H}_{10}\text{R}'$ , wherein  $n = 0, 1-3$  and  $\text{R}' = \text{H}, \text{C}_{1-3}$  alkyl  
12 e)  $(\text{CH}_2)_n\text{-heterocycle}$ , wherein  $n = 0, 1-3$  and by the term heterocyclic  
13 imidazolyl-2-yl, indolyl-3-yl, furanyl-3-yl, piridyl-3-yl, imidazolyl-  
14 3-yl are meant;  
15 f) a  $\text{-(CH}_2)_s\text{-}$  group wherein  $s = 3, 4$ , eventually OH-substituted or  
16 condensed with an aromatic group, which cyclizes with one of the two  
17 adjacent  $\text{X}_{1-6}$  groups in order to produce the side chain of proline,  
18 hydroxyproline, octahydroindol-2-carboxylic acid, tetrahydroiso-  
19 quinolinic acid;  
20 g) the side chain of a natural hydrophobic amino acid;  
21 h) the side chain of a natural hydrophilic amino acid, suitably  
22 substituted in order to render it hydrophobic;  
23 i) the side chain of non-natural hydrophobic amino acids selected from  
24 the group consisting of: norleucine, norvaline, alloisoleucine,  
25 cyclohexylglycine (Chg),  $\alpha\text{-amino-n-butyrac-acid}$  (Aba),  
26 cyclohexylalanine (Cha), aminophenylbutyric acid (Pba), mono- and di-  
27 substituted phenylalanines in ortho, meta and para positions of the  
28 benzene ring with one or more of the following groups:  $\text{C}_{1-10}$  alkyl,  
29  $\text{C}_{1-10}$  alkoxy, halogen,  $\beta\text{-2-thienylalanine}$ ,  $\beta\text{-3-thienylalanine}$ ,  $\beta\text{-2-}$   
30  $\text{furanylalanine}$ ,  $\beta\text{-3-furanylalanine}$ ,  $\beta\text{-2-piridylalanine}$ ,  $\beta\text{-3-}$   
31  $\text{piridylalanine}$ ,  $\beta\text{-4-piridylalanine}$ ,  $\beta\text{-(1-naphtyl)alanine}$ ,  $\beta\text{-(2-}$   
32  $\text{naphtyl)alanine}$ , O-alkylated serine-threonine-tyrosine-derivatives,  
33 S-alkyl cysteine, S-alkyl homocysteine, N-alkyl lysine, N-alkyl  
34 ornithine, N-alkyl 2,3 diaminopropionic acid.
- 1 3. Compounds as claimed in claim 2 wherein the side chain of a  
2 hydrophobic amino acid according to paragraph g) is the side chain of  
3 an amino acid selected from the group consisting of: glycine, alanine,

4 valine, leucine, isoleucine, methionine, phenylalanine, tyrosine,  
5 tryptophan, proline, histidine, asparagine, glutamine.

1 4. Compounds as claimed in claim 2, wherein the side chain of an  
2 hydrophilic amino acid suitably substituted according to paragraph (h)  
3 is the side chain of an amino acid selected from the group consisting  
4 of: serine, threonine, cysteine, aspartic acid, glutamic acid, t-  
5 carboxyglutamic acid, arginine, ornithine, lysine.

1 5. Compounds according to Claim 2 wherein the hydrophilic groups are  
2 chosen in the group L-Q wherein L is a chemical bond or a linear or  
3 branched C<sub>1-6</sub> alkyl-group and Q is chosen in the group consisting of:

4 i) hydroxyl, amine, guanidine, carboxyl, sulfate, phosphonate,  
5 phosphate;

6 ii) linear, branched or cyclic C<sub>1-6</sub> alkyl chain containing one or more  
7 hydroxyl, amine, guanidine, carboxyl, sulfate, phosphate;

8 iii) an aromatic group mono-, di- or tri-substituted ortho-, meta-,  
9 para-position with hydroxyl, amino, guanidine, carboxyl, sulfate,  
10 phosphate;

11 iv) a group M, OM, CONHM, NHCOM wherein M is an hydrophilic group

12 v) an hydrophilic group according to points i)-iv) protected with  
13 groups which are biologically hydrolyzed reforming an hydrophilic  
14 group.

1 6. Compounds according to Claim 5 wherein the group M is chosen in the  
2 group consisting of:

3 i) eventually substituted mono-, di-, tri-glycosidic residues;

4 ii) linear, branched or cyclic C<sub>1-6</sub> alkyl-chains, containing one or  
5 more groups hydroxyl, amine, guanidine, carboxyl, sulfate,  
5 phosphonate, phosphate.

- 1 7. Compounds of Formula (I) as claimed in claim 6, wherein the  
2 glycosidic residues are selected from the group consisting of:  
3 hexoses or pentoses of D or L series in  $\alpha$  or  $\beta$  configuration, selected  
4 from the group wherein: all C atoms bear a free or protected  
5 hydroxylic group; one or more hydroxyls are substituted by: hydrogen;  
6 an amino or acylamino group; C<sub>6</sub> of hexoses and C<sub>5</sub> of pentoses are  
7 part of a carboxylic group; and wherein the eventually present 2 or 3  
8 glycosidic units are linked by a glycosidic bond of  $\alpha$  or  $\beta$   
9 configuration.
- 1 8. Compounds of general Formula (I) according to claim 7 selected from  
2 the group consisting of: D or L ribose, D or L arabinose, D or L  
3 xylose, D or L lyxose, D or L allose, D or L altrose, D or L glucose,  
4 D or L mannose, D or L gulose, D or L idose, D or L galactose, D or L  
5 talose, D or L allulose, D or L fructose, D or L sorbose, D or L  
6 tagatose; 5-deoxy-D or L-arabinose, 2-deoxy-D or L-glucose, 2-deoxy-D  
7 or L-galactose, 2-deoxy-D or L-arabinose, 2-deoxy-D or L-ribose, D or  
8 L fucose, D or L rhamnose; D-glucosamine, D-mannosamine, D-  
9 galactosamine, daunosamine, acosamine and N-acylate derivatives thereof  
10 with lower fat acids, i.e. containing a N-formyl, acetylic,  
11 propionilic, butyric residue; glucuronic acid, galacturonic acid;  
12 cellobiose, lactose, maltose, D-lactosamine, cellotriose, maltotriose;  
13 tris(hydroxymethyl)methyl, D or L arabitol, D or L erythritol, D or L  
14 perseitol, D or L ribitol, D or L sorbitol, D or L xylitol; or those  
15 from the residue of tartaric acid, glucaric acid, gluconic acid,  
16 bycine, quinic acid, mucic acid, glucosaminic acid.
- 1 9. Compounds of general Formula (I) according to claim 1, wherein if  
2 one or both R<sub>1</sub> and R<sub>4</sub> groups are hydrophilic, both R<sub>2</sub> and R<sub>3</sub> groups

3 are hydrophobic or viceversa.

1 10. Compounds as claimed in claim 1. as hereinafter indicated:

2 i) cyclo([Asn( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 1)

3 ii) cyclo([Ser( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No.

4 2)

5 iii) cyclo ([Asn ( $\beta$ -D-2-deoxy-2-amino-Glc)-Asp-Trp-Phe-Dap-Leu]

6 cyclo (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 3)

7 iv) cyclo ( [Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)-Asp-Trp-Phe-Dap-

8 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 4)

9 v) cyclo([Nle-Asp-Trp-Phe-Dap-Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)]

10 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID 5)

11 vi) cyclo ([Asn( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

12 (2 $\beta$ -5 $\beta$ )) (SEQ ID 6)

13 vii) cyclo ( [ Ser( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo

14 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 7)

15 viii) cyclo([Asn( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

16 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 8)

17 ix) cyclo([Ser( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

18 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 9)

19 x) cyclo([Asn( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))

20 (SEQ ID No. 10)

21 xi) cyclo([Ser( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))

22 (SEQ ID No. 11)

23 xii) cyclo([Asn( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -

24 5 $\beta$ )) (SEQ ID No. 12)

25 xiii) cyclo([Ser( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -

26 5 $\beta$ )) (SEQ ID No. 13)



- 27 xiv) cyclo ([Asn( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-  
28 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 14)  
29 xv) cyclo ([Ser( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu]  
30 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 15)  
31 xvi) cyclo ([Asn(1-deoxy-sorbitol-1-yl)-Asp-Trp-Phe-Dap-Leu]cyclo  
32 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 16)  
33 xvii) cyclo ([Asn [(4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc)]-Asp-Trp-Phe-Dap-  
34 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 17)  
35 xviii) cyclo ([Asn[(4-O-( $\alpha$ -D-galactopyranosyl)- $\beta$ -D-Glc)]-Asp-Trp-Phe-  
36 Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 18)  
37 xix) cyclo ([Asn [O- $\alpha$ -D-Glc-(1-4)-O- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc]-Asp-Trp-  
38 Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 19)  
39 xx) cyclo ([Asn(D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-  
40 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 20)  
41 xxi) cyclo ([Dap[D(-)-quinyl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ  
42 ID No. 21)  
43 xxii) cyclo ([Dap[D-gluconyl]-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ )) (SEQ  
44 ID No. 22)  
45 xxiii)cyclo ([Dap[D-glucuryl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ  
46 ID No. 23)  
47 xxiv) cyclo([Dap(2-sulfo-benzoyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
48 (SEQ ID No. 24)  
49 xxv) cyclo ([Asn(4-sulfo-phenyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
50 (SEQ ID No. 25)  
51 xxvi) cyclo ([Asn( $\beta$ -L-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID  
52 No. 26)  
53 xxvii) cyclo ([Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-

- 54 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 27)
- 55 xxviii) cyclo ([Asn( $\beta$ -D-2-deoxy-mannopyranos-2-yl)-Asp-Trp-Phe-Dap-
- 56 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 28)
- 57 xxix) cyclo ([Asn(D-2-deoxy-galactopyranos-2-yl)-Asp-Trp-Phe-Dap-
- 58 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 29)
- 59 xxx) cyclo ([Asn( $\beta$ -D-xylopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))
- 60 (SEQ ID No. 30)
- 61 xxxi) cyclo ([Asn(3-sulfo-propionyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -
- 62 5 $\beta$ )) (SEQ ID No. 31)
- 63 xxxii) cyclo ([Dap(Lysyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID
- 64 No. 32)
- 65 xxxiii) cyclo ([Dap(Arginyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID
- 66 No. 33)
- 67 xxxiv) cyclo ([Dap(4-O- $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]
- 68 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 34)
- 69 xxxv) cyclo ([Asn(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc)-Asp-Trp-Phe-
- 70 Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 35).

1 11. Pharmaceutical compositions containing as active principle  
2 compounds of general Formula (I) as claimed in claim 1, combined to  
3 suitable carriers.

1 12. Pharmaceutical compositions according to claim 11 for use as  
2 tachykinins antagonists.

1 13. Pharmaceutical compositions as claimed in claim 12 for treatment  
2 of arthrytis, asthma, inflammations, tumoral growth, gastrointestinal  
3 hypermotility, Huntington's disease, neuritis, neuralgia, hemicrania,  
4 hypertension, urinary incontinence, urticaria, symptoms from carcinoid  
5 syndrome, flu and cold.

1 14. Methods for treatment of arthrytis, asthma, inflammations, tumoral  
2 growth, gastrointestinal hypermotility, Huntington's desease,  
3 neuritis, neuralgia, hemicrania, hypertension, urinary incontinence,  
4 urticaria, symptoms from carcinoid syndrome, flu and cold, all  
5 conditions in which doses comprised between 0.1 and 10 mg/Kg of body  
6 weight of active principle consisting of the products of Formula (I).  
7 according to claim 1. are administered to the patient.

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## INTERNATIONAL SEARCH REPORT

In national Application No

PCT/EP 96/01028

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07K7/22 C07K7/56 C07K7/64 C07K9/00 A61K38/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 21227 (MENARINI ET AL.) 28 October 1993 cited in the application see the whole document ---	1-9, 11-14
Y	INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, vol. 44, no. 2, August 1994, COPENHAGEN DK, pages 105-111, XP000456585 G HÖLZEMANN ET AL.: "Cyclic hexapeptide NK-2 antagonists" see the whole document ---	1-9, 11-14
-/-		

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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(21) International Application Number: PCT/EP96/01028 (22) International Filing Date: 11 March 1996 (11.03.96) (30) Priority Data: FI95A000044          13 March 1995 (13.03.95)          IT (71) Applicant (for all designated States except US): A. MENARINI INDUSTRIE FARMACEUTICHE RIUNITE S.R.L. [IT/IT]; Via Sette Santi, 3, I-50131 Florence (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): ARCAMONE, Federico [IT/IT]; Via 4 Novembre, 26, I-20014 Nerviano (IT). MAGGI, Carlo, Alberto [IT/IT]; Via Michelazzi, 43, I- 50100 Florence (IT). QUARTARA, Laura [IT/IT]; Viale Os- imo, 385, I-52037 Sansepolcro (IT). GIANNOTTI, Danilo [IT/IT]; Via Roma, 128, I-55011 Altopascio (IT). (74) Agent: GERVASI, Gemma; Notarbartolo & Gervasi S.r.l., Viale Bianca Maria, 33, I-20122 Milan (IT).		(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(54) Title: BICYCLIC TACHYKININS ANTAGONISTS, PREPARATION THEREOF AND THEIR USE IN PHARMACEUTICAL COMPOSITION			
(57) Abstract			
This invention relates to novel compounds of general formula (I) and to pharmaceutical compositions containing them.			
<div style="text-align: center;"> <math display="block">  \begin{array}{ccccc}  R_1 &amp; &amp; &amp; &amp; R_2 \\  &amp; \diagdown &amp; &amp; \diagup &amp; \\  &amp; CH &amp; -X_2- &amp; CH &amp; -X_3- &amp; CH \\  &amp;   &amp; &amp;   &amp; &amp;   \\  &amp; &amp; &amp; (CH_2)_n &amp; &amp; \\  &amp; &amp; &amp;   &amp; &amp; \\  &amp; &amp; &amp; Y &amp; &amp; \\  &amp; &amp; &amp;   &amp; &amp; \\  &amp; &amp; &amp; (CH_2)_m &amp; &amp; \\  &amp; &amp; &amp;   &amp; &amp; \\  R_4 &amp; \diagup &amp; &amp; \diagdown &amp; &amp; R_3 \\  &amp; CH &amp; -X_6- &amp; CH &amp; -X_5- &amp; CH \\  &amp;   &amp; &amp;   &amp; &amp;   \\  &amp; &amp; &amp; &amp; &amp;   \end{array}  </math> </div> <div style="text-align: right;">(I)</div>			

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- 1 -

BYCYCLIC TACHYKININS ANTAGONISTS, PREPARATION THEREOF AND  
THEIR USE IN PHARMACEUTICAL COMPOSITION

Field of the Invention

This invention relates to novel bi-cyclic compounds useful in  
5 pharmaceutical compositions as tachykinins antagonists, and to  
pharmaceutical compositions containing them.

Background of the invention

The receptor NK<sub>2</sub> of tachykinins is widely expressed in the  
peripheral nervous system of Mammalia. One of the several effects  
10 caused by the selective stimulation of the receptor NK<sub>2</sub> is the  
contraction of the smooth muscles. Therefore, antagonists of the  
receptor NK<sub>2</sub> can be considered agents able to control the  
hypercontraction of the smooth muscles in any pathological condition in  
which the release of the tachykinins contributes to the rise of the  
15 corrispondent disorder. In particular, the bronchospastic component of  
asthma, cough, pulmonary irritations and local spasms of the urinary  
bladder and of the ureter during cystitis, infections and renal colics  
can be considered conditions in which the administration of receptor  
NK<sub>2</sub> antagonists can be effective (A.L. Magnan et al. *Neuropeptides*,  
20 1993, 24, 199). Compounds which act as antagonists of the tachykinins,  
and in particular of the neurokinin A, are well-known in Literature.  
Among them, the cyclic compounds (B. J. Williams et al. *J. Med. Chem.*,  
1993, 36, 2) are of particular interest. Lipophily has been defined as  
an essential requirement in order to have an intensive antagonist  
25 activity to the receptor NK<sub>2</sub> of the tachykinins of a series of cyclic  
pseudopeptides (L. Quartara et al. *J. Med. Chem.*, 1994, 27) and

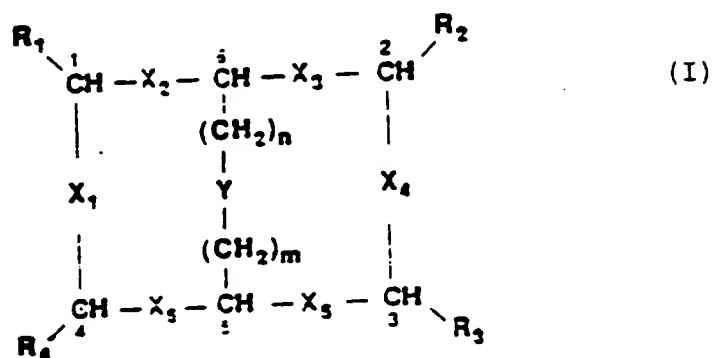
- 2 -

particularly in case of bicyclic hexapeptides. WO/ 93/21227).  
 Surprisingly it has been now found that products structurally similar  
 to those described above, but in which, however, at least one  
 hydrophilic group is present, not only keep their high affinity *in*  
 5 *vitro*, but also show an increase in the pharmacological activity *in*  
*vivo* if compared to the correspondent compounds which do not contain  
 any hydrophilic group.

This is even more surprising if it is taken into account that  
 monocyclic peptides having antagonist properties which are similar to  
 10 those of the tachykinins do not show any increase in the  
 pharmacological activity when hydrophilic groups are introduced onto  
 the structure of the cycle [Int. J. Peptide Protein Res. (1984), 44:2,  
 105-111].

### Summary

15 This invention relates to novel compounds of the general formula (I):



wherein:

$X_1, X_2, X_3, X_4, X_5$ , and  $X_6$ , same or different from one another,  
 represent a - NR'CO- or a -CONR'- group, wherein R' is H or C<sub>1-3</sub>  
 alkyl;



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Y represents a group selected from -NRCO-, -CONR-, or -SS-

wherein R is H or C<sub>1-3</sub> alkyl;

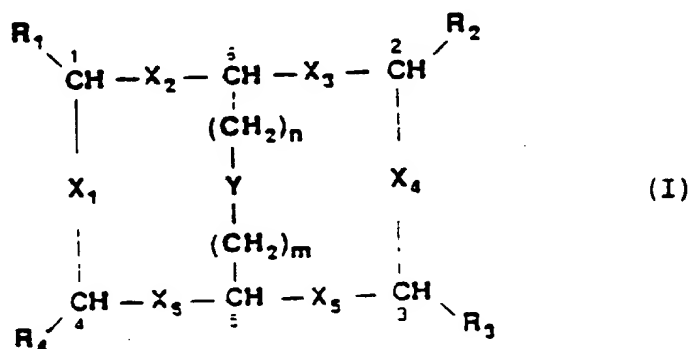
at least one of the R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> groups, same or different from one another, is hydrophilic and the remaining groups are hydrophobic;

5 m and n, same or different from one another, are each an integer number from 1 to 4;

and to pharmaceutical compositions containing them.

#### Detailed description of the Invention

The present invention relates to novel compounds having the general  
10 formula (I)



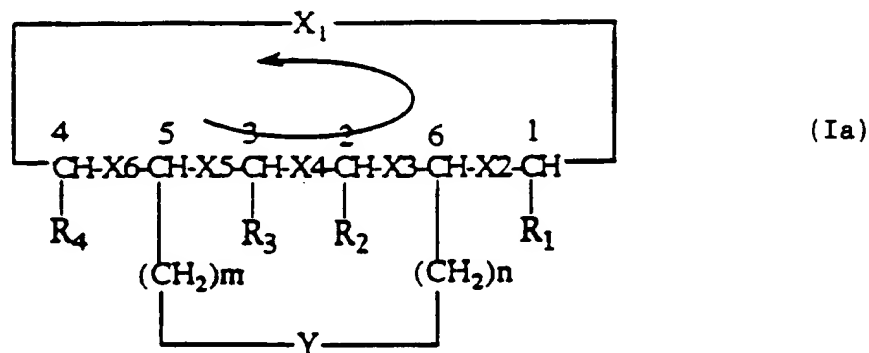
wherein

X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>; Y, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, m and n groups are as defined above;

processes for the preparation thereof and pharmaceutical compositions containing them.

The formula (I) as reported above is considered the one giving the  
15 best representation of the real spatial structure of the bicyclic peptide according to the invention. However also the following Formula (Ia) (which chemically speaking is identical to Formula (I)) is given

in order to simplify the understanding of the compounds described hereinafter and in the Examples with their chemical name in particular in so far as the groups  $X_{1-6}$  and Y are concerned.



The groups  $X_{1-6}$  and Y are in fact defined according to the aminoacid-sequence from the formal N- to the C-terminus of the peptide as they are represented in the linear structure, therefore reading Formula (Ia) no problem arises in the understanding of the linear structure as reported in the Examples.

As it can be seen, the compounds of formula (I) as described above present chiral centers: it is understood that this invention relates also to the several enantiomers.

More particularly the hydrophobic groups can be separately selected from the following:

a) groups  $C_nH_{2n+1}$  wherein  $n = 0, 1-4$

b) linear- or branched alkyl groups corresponding to  $C_nH_{2n}-U-W$  wherein  $n = 1-4$ ;  $U = O, COO, CONH, S$  and  $W = \text{alkyl-, aryl or alkylaryl-group}$  containing from 1 to 15 carbon atoms

c)  $(CH_2)_n - C_6H_3 - A - B$  wherein  $n = 0, 1-3$ ; A and B, placed in any of the ortho, meta or para positions, same or different from one another, represent H, halogen, OR, NHR,  $NR_2$ ,  $CH_3$ , SR wherein R is an alkyl-, aryl- or alkylaryl-group with less than 10 C atoms

d)  $(CH_2)_n - C_6H_{10} - R'$ , wherein  $n = 0, 1-3$  and  $R' = H, C_{1-3} \text{ alkyl}$

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e)  $(CH_2)_n$  -heterocycle. wherein  $n = 0, 1-3$  and for heterocycle it is meant: imidazolyl-2-yl. indolyl-3-yl. furanyl-3-yl. pyridyl-3-yl. imidazolyl-3-yl

f) a  $-(CH_2)_s-$  group. wherein  $s = 3, 4$ , eventually OH-substituted or condensed with an aromatic group, which cyclizes with one of the two adjacent  $X_{1-6}$  groups in order to produce the side chain of proline, hydroxyproline, octahydroindol-2-carboxylic acid, tetrahydroisoquinolinic acid

g) the side chain of a natural hydrophobic amino acid

h) the side chain of a natural hydrophilic amino acid, suitably substituted in order to render it hydrophobic

i) the side chain of non-natural hydrophobic amino acids selected from the group consisting of: norleucine, norvaline, alloisoleucine, cyclohexylglycine (Chg),  $\alpha$ -amino-n-butyric acid (Aba),

cyclohexylalanine (Cha), aminophenylbutyric acid (Pba), phenylalanines mono- and di- substituted in the ortho, meta and para positions of the benzene ring with one or more of the following groups:  $C_{1-10}$  alkyl,  $C_{1-10}$  alkoxy, halogen,  $\beta$ -2-thienylalanine,  $\beta$ -3-thienylalanine,  $\beta$ -2-furanylalanine,  $\beta$ -3-furanylalanine,  $\beta$ -2-piridylalanine,  $\beta$ -3-piridylalanine,  $\beta$ -4-piridylalanine,  $\beta$ -(1-naphtyl)alanine,  $\beta$ -(2-naphtyl)alanine, O-alkylated serine- threonine- tyrosine-derivatives, S-alkyl cysteine, S-alkyl homocysteine, N-alkyl lysine, N-alkyl ornithine, N-alkyl 2,3 diaminopropionic acid.

More particularly, the side chain of a hydrophobic amino acid according to paragraph (g) is the side chain of an amino acid selected from the group consisting of: glycine, alanine, valine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan, proline, histidine.

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asparagine, glutamine.

The side chain of a hydrophilic amino acid, suitably substituted in order to render it hydrophobic according to paragraph (h) is the chain of an amino acid selected from the group consisting of: serine,  
5 threonine, cysteine, aspartic acid, glutamic acid, t-carboxyglutamic acid, arginine, ornithine, lysine.

Preferably, the hydrophilic groups are selected from L-Q group, wherein L is a chemical bond or a linear or branched C<sub>1-6</sub>-alkyl residue and Q is a hydrophilic group. Preferably Q is selected from  
10 the group consisting of: guanidine, amine, M, OM, -CO-NH-M, -NH-CO-M, an aromatic group which has been mono-, di- or tri-substituted in ortho, meta, para positions with M or OM groups, wherein M is a hydrophilic group.

With the term "hydrophilic group", for Q and M, it is preferably  
15 meant:

- i) eventually substituted mono-, di-, tri-glycosidic residues;
- ii) C<sub>1-6</sub> linear or cyclic alkyl chains comprising one or more polar groups;
- iii) hydroxyl, amine, guanidine, carboxyl, sulfate, phosphonate,  
20 phosphate;
- iv) residues bearing substituted hydrophilic groups which in biologic environment are hydrolysed, re-establishing the hydrophilic function.

As far as the definition according to paragraph (i) hereinabove is  
25 concerned, the following structures are preferably meant:

hexoses or pentoses of the D or L series in  $\alpha$  or  $\beta$  configuration, selected from the group wherein: all C atoms bear a free or protected

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hydroxylic group; one or more hydroxyls are substituted by: hydrogen,  
an amino or acylamino group;  $C_6$  of hexoses and  $C_5$  of pentoses are part  
of a carboxylic group; and wherein the eventually present 2 or 3  
glycosidic units are linked by a glycosidic bond of  $\alpha$  or  $\beta$   
5 configuration.

Specific examples of glycosidic groups as defined above are: D or L  
ribose, D or L arabinose, D or L xylose, D or L lyxose, D or L allose,  
D or L altrose, D or L glucose, D or L mannose, D or L gulose, D or L  
idose, D or L galactose, D or L talose, D or L allulose, D or L  
10 fructose, D or L sorbose, D or L tagatose; 5-deoxy-D or L-arabinose,  
2-deoxy-D or L-glucose, 2-deoxy-D or L-galactose, 2-deoxy-D or L-  
arabinose, 2-deoxy-D or L-ribose, D or L fucose, D or L ramnose; D-  
glucosamine, D-mannosamine, D-galactosamine, daunosamine, acosamine  
and N-acylate derivates thereof with lower fatty acids, i.e. having a  
15 N-formylic, acetylic, propionilic, butyric residue; glucuronic acid,  
galacturonic acid, cellobiose, lactose, maltose, D-lactosamine,  
cellotriose, maltotriose and protected derivates thereof.

The definition according to paragraph (ii) hereinabove applies to  
chains deriving from a polyol-residue, such as  
20 tris(hydroxymethyl)methyl, D or L arabitol, D or L erythrol, D or L  
galactytol, meso-inositol, D or L mannitol, D or L perseitol, D or L  
ribitol, D or L sorbitol, D or L xylitol; or those deriving from the  
residue of tartaric acid, glucaric acid, gluconic acid, bycine, quinic  
acid, mucic acid, glucosaminic acid.

25 Among the products of formula (I) as above indicated, the products  
wherein if one or both  $R_1$  and  $R_4$  groups are hydrophilic, both  $R_2$  and  
 $R_3$  groups are hydrophobic and viceversa, are particularly preferred.

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Compounds of formula (I) object of the present invention can be synthesized by the various techniques known in Literature, see e.g. M. Bodansky, "Peptide Chemistry", Springer-Verlag, 1988.

For example by means of in solution synthesis of the linear peptidic chain through subsequent coupling of suitably activated N-protected amino acids to an amino acid or to a C-protected peptidic chain, with isolation of the intermediates, subsequent selective de-protection of the C- and N-terminal chains, cyclization in polar organic solvents in diluted solution, hence selective de-protection of the side chains and at last cyclization of the same in polar organic solvents in diluted solution. The hydrophilic residue can be introduced both as protected amino acid derivative during the peptidic chain synthesis and by means of conjugation to the already formed peptide, as widely disclosed in Literature. Similarly a synthesis in solid phase of the peptidic chain from the C-terminal end to the N-terminal one on a insoluble polymeric support, the cyclization in solid phase between the previously de-protected side chains, the subsequent detachment from the polymeric support by means of hydrolysis in anhydrous hydrofluoric acid containing the suitable scavengers or in trifluoroacetic acid containing the suitable scavengers or in aqueous bases and the cyclization of the monocyclic peptide in polar organic solvents in diluted solution, can be used for the preparation. The hydrophilic residue being introduced according to the above disclosed indications. According to a particular preparation method, the desired product can be obtained in solid phase using the 2-chlorotrytil resin (Barlos et al., Int. J. Peptide Protein Res., 37, 513-520, 1991) substituted with a protected amino acid having the Fmoc group at the N-terminal end;

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preferably the amino acid directly bond to the resin is the one having the  $R_1$  or  $R_3$  side chain. After the other amino acids being introduced in the sequence, the peptide is detached from the resin with diluted acetic acid and a first cyclization is performed between the free C-terminal and N-terminal end by means of the conventional classic synthesis methods. Subsequently, the amino acid side chains are de-protected in position 5 and 6, for example with trifluoroacetic acid, and way is given to the second cyclization.

Other synthetic ways are anyway possible and largely described in Literature as above mentioned.

The compounds of formula (I) as above indicated have revealed to be powerful antagonists of the receptor  $NK_2$  of the tachykinins, and hence may be administered in doses which are not higher than those required for the known products.

They can be therefore indicated for the treatment of arthritis, asthma, inflammations, tumoral growth, gastro-intestinal hypermotility, Huntington's disease, neurites, neuralgia, hemicrania, hypertension, urinary incontinence, urticaria, symptoms from carcinoid disease, flu and colds.

The compounds of formula (I) object of the present invention are suitable for the parenteral, oral, inhalatory and sublingual administration for therapeutical purposes to the superior animals and to the humans, achieving pharmacological effects according to the above described features. For parenteral administrations (endovenous, intramuscular and intradermic) sterile solutions or lyophilized chemical preparations are used. For nasal, inhalatory and sublingual administrations, according to the particular instance,

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aqueous solutions, aerosol preparations or capsules are used.

The doses of active principle in the above compositions can be comprised between 0.1 and 10 mg/kg of body weight.

EXAMPLE 1.

5 Preparation of cyclo([Asn( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 1) compound of formula (I) wherein  $Y=X_1=X_2=X_3=X_4=X_5=X_6=-$ CO-NH-;  $R_1=-CH_2-CH(CH_3)_2$ ;  $R_2=-CH_2-C_6H_5$ ,  $R_3=-CH_2$ indolyl-3-yl,  $R_4=-CH_2-CO-NH-(\beta$ -D-Glc);  $m=n=1$  and the carbon atoms  $C_1, C_2, C_3, C_4, C_5, C_6$  have L configuration].

10 a) synthesis of the linear peptide H-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-Asp(OtBu)-Trp-Phe-Dap(Boc)-Leu-OH.

1 g of 2-chlor trityl resin (1.6 mmol/g. Novabiochem) is functionalized with Fmoc-Leu-OH (0.6 eqs.) as described by Barlos et al., Int. J. Peptide Protein Res., 1991, 37, 513-520. The substitution  
15 degree of the resin is determined by dosing the group Fmoc, and it is equal to 0.364 meq/g. The subsequent 4 amino acids are coupled as free acids using an excess 3 of amino acid and HOBt (4 eqs.) and DCC (3 eqs.) as activators with reaction times of 1 hour. In the following order: Fmoc-Dap(Boc)-OH, Fmoc-Phe-OH, Fmoc-Trp-OH, Fmoc-Asp(OtBu)-OH  
20 are added. The last amino acid is coupled as Fmoc-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp (Christiansen-Brans et al., J.Chem.Soc. Perkin Trans. I, 1993, 1461-1471), 2 eqs., with HOBt (2 eqs.) as activator, for 3h.

After the de-protection of the group Fmoc, the detachment from the resin is performed, suspending it in 10 mL of a mixture of AcOH, TFE,  
25 DCM (1/1/8, v/v) at room temperature for 0.5 h. Thereafter the solvent is evaporated under vacuum at 30°C, it is again mixed with water and it is lyophilized. Yield in raw product: 405 mg (90 %). Title HPLC: 70



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%. FAB-MS:  $[M+H]^+ = 1266$ ;  $t_R$ : 14.7 min.

b) Synthesis of the bicyclic product cyclo([Asn((Ac<sub>4</sub>O)- $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (compound 2).

The linear raw product is cyclized in 1 mM solution in DMF, at 4°C, with 1 eq. of PyBOP and 1.2 eqs. of DIEA for 1 h. The mixture is dried and purified in HPLC obtaining 156 mg of the pure product (yield 39 %). Title HPLC: >99 %. FAB-MS:  $[M+H]^+ = 1248$ ;  $t_R$ : 18.4 min.

The monocyclic product is de-protected by solving it in 15 ml of TFA containing water at 10 %. After 0.5 h. the mixture is diluted in water and it is lyophilized. The residue is dissolved in 1 mM solution in DMF, the solution is brought to 0°C and 1 eq. of PyBOP and 1.2 eqs. of DIEA are added. After 5 h. it is dried and purified in HPLC. Yield 45 % (70 mg). Title HPLC > 99 %. FAB-MS:  $[M+H]^+ = 1074$ ;  $t_R$ : 13.5 min.

c) Synthesis of the bicyclic product cyclo ([Asn( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))

70 mg of tetraacetylate product are dissolved in anhydrous methanol in 5 mM solution. The solution is brought to -20°C and a 1 mM solution of sodium methylete in methanol is added to achieve pH = 11. After 10' acetic acid is added to achieve neutral pH. high dilution with water and lyophilization follow. Yield 60 %. Title HPLC: 98 %. FAB-MS:  $[M+H]^+ = 906$ ;  $t_R$ : 9.3 min.

#### EXAMPLE 2

Preparation of cyclo([Ser( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 2) [compound of Formula (I) wherein:  $Y=X_1=X_2=X_3=X_4=X_5=X_6=-CO-NH-$ ;  $R_1=-CH_2-CH(CH_3)_2$ ;  $R_2=-CH_2-C_6H_5$ ;  $R_3=-CH_2-indolyl-3-yl$ ;  $R_4=-CH_2-O-(\beta-D-Glc)$ ;  $m = n = 1$  and  $C_1, C_2, C_3, C_4, C_5, C_6$  carbon atoms have L configuration].

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a) synthesis of linear peptide H-Ser[(Bz<sub>4</sub>O)-β-D-Glc]-Asp(OtBu)-Trp-Phe-Dap(Boc)-Leu-OH.

The same procedure which has been used for Example 1), paragraph a), is utilized here till the addition of the last amino acid, which is coupled as Fmoc-Ser[(Bz<sub>4</sub>O)-β-D-Glc]-OPfp (obtained by the procedure which has been described by Vargas-Berenguel et al., J. Chem. Soc. Perkin Trans. I, 1994, 2615, 2619).

The detachment occurs as described above, in Example 1). Yield in raw product: 450 mg (83 %). Title HPLC: 93 %. FAB-MS: [M+H]<sup>+</sup> = 1487; t<sub>r</sub>: 20.8 min.

b) Synthesis of bicyclic product cyclo([Ser[(Bz<sub>4</sub>O)-β-D-Glc]-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)).

The linear raw product is cyclized in 1mM solution in DMF, at 4°C, with 1 eq. of PyBOP and 1.2 eqs. of DIEA for 1 h. The mixture is dried and purified in HPLC, obtaining 0.16 g of pure product (yield 35 %). Title HPLC: >99 %. FAB-MS: [M+H]<sup>+</sup> = 1469; t<sub>r</sub>: 25.3 min.

The monocyclic product is de-protected by liquefying it in 10 mL of TFA containing water at 10 %. After 0.5 h the mixture is diluted in water and it is lyophilized. The residue is dissolved in 1mM solution in DMF, the solution is brought to 0°C and 1 eq. of PyBOP and 1.2 eqs. of DIEA are added. After 24 h it is dried and purified in HPLC. Yield 63 mg (45 %). Title HPLC: >99 %. FAB-MS: [M+H]<sup>+</sup> = 1295; t<sub>r</sub>: 21.6 min.

c) Synthesis of bicyclic product cyclo([Ser(β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)).

20 mg of tetrabenzoylate product are dissolved in anhydrous methanol in 5mM solution. The solution is brought to -20°C and a 1mM solution of sodium methylate in methanol is added to achieve pH = 11. After 1.5

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h acetic acid is added to achieve neutral pH. high dilution with water and lyophilization follow. Yield: 6.5 mg (48 %). Title HPLC: > 99 %. FAB-MS:  $[M+H]^+ = 878$ ;  $t_R$ : 9.6 min.

By similar procedures, the following compounds have been obtained:

5   EXAMPLE 3

cyclo([Asn( $\beta$ -D-2-deoxy-2-amino-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 3) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-amino-Glc) and the other substituents are as defined in Example 1].

10   EXAMPLE 4

cyclo ([Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 4) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-acetamido-Glc) and the other substituents are as defined in Example 1].

15   EXAMPLE 5

cyclo ([Nle-Asp-Trp-Phe-Dap-Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 5) [compound of Formula I) wherein  $R_1 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-acetamido-Glc),  $R_4 = -(CH_2)_3-CH_3$ ] and the other substituents are as defined in Example 1].

20   EXAMPLE 6

cyclo([Asn( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 6) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-ribofuranosyl) and the other substituents are as defined in Example 1].

25   EXAMPLE 7

cyclo([Ser( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 7) [compound of Formula I) wherein  $R_4 = -CH_2-O-(\beta$ -D-

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ribofuranosyl), and the other substituents are as defined in Example 1].

## EXAMPLE 8

cyclo ([Asn ( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
5 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 8) [compound of Formula I) wherein  $R_4$  = -CH<sub>2</sub>-CO-  
NH-( $\beta$ -L-arabinofuranosyl) and the other substituents are as defined in  
Example 1].

## EXAMPLE 9

cyclo ([Ser ( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
10 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 9) [compound of Formula I) wherein  $R_4$  = -CH<sub>2</sub>-O-( $\beta$ -  
L-arabinofuranosyl) and the other substituents are as defined in  
Example 1].

## EXAMPLE 10

cyclo([Asn( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
15 (SEQ ID 10) [compound of Formula I) wherein  $R_4$  = -CH<sub>2</sub>-CO-NH-( $\beta$ -D-  
mannopyranosyl) and the other substituents are as defined in Example  
1].

## EXAMPLE 11

cyclo([Ser( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
20 (SEQ ID No. 11) [compound of Formula I) wherein:  $R_4$  = -CH<sub>2</sub>-O-( $\beta$ -D-  
mannopyranosyl) and the other substituents are as defined in Example  
1].

## EXAMPLE 12

cyclo ([Asn ( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
25 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 12) [compound of Formula I) wherein  $R_4$  = -CH<sub>2</sub>-CO-  
NH-( $\beta$ -D-galactopyranosyl) and the other substituents are as defined in  
Example 1].

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## EXAMPLE 13

cyclo([Ser( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )  
(SEQ ID No. 13) [compound of Formula I) wherein  $R_4 = -CH_2-O-(\beta$ -D-  
galactopyranosyl) and the other substituents are as defined in Example  
5 1].

## EXAMPLE 14

cyclo ([Asn( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 14) [compound of Formula I) wherein  $R_4 = -CH_2-CO-$   
NH-( $\beta$ -D-glucuronopyranosyl) and the other substituents are as defined  
10 in Example 1].

## EXAMPLE 15

cyclo( [Ser( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 15) [compound of Formula I) wherein  $R_4 = -CH_2-O-$   
( $\beta$ -D-glucuronopyranosyl) and the other substituents are as defined in  
15 Example 1].

## EXAMPLE 16

cyclo ([Asn(1-deoxy-sorbitol-1-yl)-Asp-Trp-Phe-Dap-Leu] cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID 16) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-$   
(1-deoxy-sorbitol-1-yl) and the other substituents are as defined in  
20 Example 1].

## EXAMPLE 17

cyclo ([Asn[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc]]-Asp-Trp-Phe-Dap-Leu]cyclo-  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 17) [compound of Formula I) wherein  $R_4 = -CH_2-CO-$   
NH-[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc]] and the other substituents are as defined  
25 in Example 1].

## EXAMPLE 18

cyclo([Asn[4-O-( $\alpha$ -D-galactopyranosyl)- $\beta$ -D-Glc]-Asp-Trp-Phe-Dap-

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Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 18) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-[4-O( $\beta$ -D-galactopyranosyl)- $\beta$ -D-Glc]] and the other substituents are as defined in Example 1].

## EXAMPLE 19

5 cyclo ([ Asn [ 0- $\alpha$ -D-Glc-( 1-4 )-0- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc]-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 19) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-[0- $\alpha$ -D-Glc-(1-4)-0- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc) and the other substituents are as defined in Example 1].

## EXAMPLE 20

10 cyclo([Asn(D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 20) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-(D-2-deoxy-glucopyranos-2-yl) and the other substituents are as defined in Example 1].

## EXAMPLE 21

15 cyclo ([Dap[D(-)-quinyl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 21) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-NH-[D(-)-quinyl], and the other substituents are as defined in Example 1].

## EXAMPLE 22

cyclo ([Dap[D-gluconyl]-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))  
20 (SEQ ID No. 22) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-NH-(D-gluconyl) and the other substituents are as defined in Example 1].

## EXAMPLE 23

cyclo ([Dap[D-glucuryl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
(SEQ ID No. 23) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-NH-(D-glucuryl) and the other substituents are as defined in Example 1].  
25

## EXAMPLE 24

cyclo ([Dap(2-sulfo-benzoyl)-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ ))

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(SEQ ID No. 24) [compound of Formula I) wherein:  $R_4 = -CH_2-NH-CO-C_6H_4-SO_3H$  and the other substituents are as defined in Example 1].

## EXAMPLE 25

cyclo ([Asn (4-sulfo-phenyl)-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ ))

5 (SEQ ID No. 25) [compound of Formula I) wherein  $R_4 = CH_2-CO-NH-C_6H_4-SO_3H$  and the other substituents are as defined in Example 1].

## EXAMPLE 26

cyclo([Asn( $\beta$ -L-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 26)

[compound of Formula I) wherein  $R_4 = -CH_2-CO-NH(\beta$ -L-Glc) and the other  
10 substituents are as defined in Example 1].

## EXAMPLE 27

cyclo([Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-

Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 27) [compound of formula I) wherein  $R_4$   
=  $-CH_2-CO-NH-(D-2-deoxy-glucopyranos-2-yl)$  and the other substituents

15 are as defined in Example 1].

## EXAMPLE 28

cyclo ([Asn(D-2-deoxy-mannopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]-

cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 28) [compound of formula I) wherein  $R_4 = -$   
 $CH_2-CO-NH-(D-2-deoxy-mannopyranos-2-yl)$  and the other substituents are  
20 as defined in Example 1].

## EXAMPLE 29

cyclo ([Asn(D-2-deoxy-galactopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]-

cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 29) [compound of formula I) wherein  $R_4 = -$   
 $CH_2-CO-NH-(D-2-deoxy-galactopyranos-2-yl)$  and the other substituents  
25 are as defined in Example 1].

## EXAMPLE 30

cyclo ([Asn( $\beta$ -D-xylopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))

- 18 -

(SEQ ID No. 30) [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta-D\text{-xylo-pyranosyl})$  and the other substituents are as defined in Example 1].

## EXAMPLE 31

5   cyclo ([Asn(3-sulfo-propionyl)-Asp-Trp-Phe-Dap-Leu]cyclo-(2 $\beta$ -5 $\beta$ ))  
(SEQ ID 31) [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(3\text{-sulfo-propionyl})$  and the other substituents are as defined in Example 1].

## EXAMPLE 32

cyclo ([Dap(Lysyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 32)  
10 [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(Lysyl)$  and the other substituents are as defined in Example 1].

## EXAMPLE 33

cyclo ([Dap(Arginyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 33)  
[compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(Arginyl)$  and the  
15 other substituents are as defined in Example 1].

## EXAMPLE 34

cyclo ([Dap(4-O- $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo-  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 34) [compound of formula I) wherein  $R_4 = -CH_2-CO-$   
NH-(4-O- $\beta$ -D-galactopyranosyl) and the other substituents are as  
20 defined in Example 1].

## EXAMPLE 35

cyclo ([Asn(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-  
Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 35) [compound of formula I) wherein  $R_4 =$   
-CH<sub>2</sub>-CO-NH-(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc) and the other  
25 substituents are as defined in Example 1].

BIOLOGICAL ACTIVITY

The capability of the compounds of the present invention to interact



In                      tonal Application No                     

PCT/EP 96/01028

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9321227	28-10-93	BG-A- 99110	29-09-95
		CZ-A- 9402542	12-07-95
		EP-A- 0636146	01-02-95
		FI-A- 944838	14-10-94
		HU-A- 70189	28-09-95
		JP-T- 8500331	16-01-96
		NO-A- 943861	13-10-94
		SK-A- 124294	11-07-95
		ZA-A- 9302644	22-10-93
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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/01028

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEMICAL ABSTRACTS, vol. 122, no. 5, 30 January 1995 Columbus, Ohio, US; abstract no. 46372p, C A MAGGI ET AL.: "MEN 10, 627, a novel polycyclic peptide antagonist of tachykinin NK-2 receptors" page 114; XP002007657 see abstract &amp; J PHARM EXP THER, vol. 271, no. 3, 1994, pages 1489-1500,</p> <p>-----</p>	1-14

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 96/01028

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 14 refers to a method of treatment of the human body the search was carried out and based on the alleged effects of the products.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

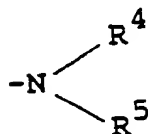
This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

4. A compound of claim 3, wherein  
 R<sup>1</sup> is hydrogen or acyl,  
 R<sup>2</sup> is hydrogen, acyl, carbamoyl(lower)alkyl, carboxy(lower)alkyl or esterified carboxy(lower)alkyl,  
 R<sup>3</sup> is ar(lower)alkyl, a group of the formula:



wherein R<sup>4</sup> is hydrogen, lower alkyl, hydroxy(lower)alkyl or acyloxy(lower)alkyl, and  
 R<sup>5</sup> is aryl, ar(lower)alkyl or haloar(lower)alkyl, or  
 R<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-condensed lower alkylene, or

a group of the formula:  
 -OR<sup>6</sup>

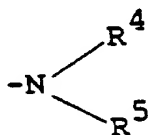
wherein R<sup>6</sup> is aryl, lower alkyl, ar(lower)alkyl, haloar(lower)alkyl or pyridyl(lower)alkyl.

5. A compound of claim 4, wherein

R<sup>1</sup> is hydrogen, carbamoyl, lower alkoxycarbonyl, lower alkanoyl, ar(lower)alkoxycarbonyl, carbamoyl(lower)-  
 alkanoyl, lower alkoxalyl, di(lower)alkylamino(lower)alkanoyl, N-ar(lower)alkyl-N-lower alkoxycarbonylamino-  
 (lower)alkanoyl, tetrazolyl(lower)alkanoyl, carboxy(lower)alkanoyl, hydroxy(lower)alkanoyl, morpholinecar-  
 bonyl, N-lower alkylcarbamoyl, lower alkanoylaminothiazolyl(lower)alkanoyl, lower alkanoylaminothiazolyl-  
 (lower)alkanoyl having lower alkoxycarbonylamino or lower alkanylamino on the alkanoyl moiety, carboxy-  
 (lower)alkylamino(lower)alkanoyl, ar(lower)alkylamino(lower)alkanoyl or N-lower alkoxycarbonyl-N-lower  
 alkoxycarbonyl(lower)alkylamino(lower)alkanoyl,

R<sup>2</sup> is hydrogen, lower alkanoyl, arenesulfonyl, carbamoyl(lower)alkyl, carboxy(lower)alkyl or lower  
 alkoxycarbonyl(lower)alkyl,

R<sup>3</sup> is ar(lower)alkyl,  
 a group of the formula :



wherein R<sup>4</sup> is hydrogen, lower alkyl, hydroxy(lower)alkyl or ar(lower)alkoxycarbonyloxy(lower)-  
 alkyl, and  
 R<sup>5</sup> is aryl, ar(lower)alkyl or haloar(lower)alkyl, or

R<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-condensed lower alkylene, or  
 a group of the formula :

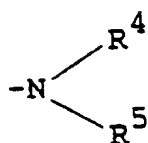
-OR<sup>6</sup>

wherein R<sup>6</sup> is as defined in claim 4, and

A is one or two amino acid residue(s) derived from amino acid(s) selected from glutamine, serine,  
 asparagine, glutamic acid, threonine, lysine, histidine, β-aspartic acid, ornithine, glycine, tyrosine, trypto-  
 phan, hydroxyproline, pyroglutamic acid, β-alanine, N<sup>5</sup>,N<sup>5</sup>-di(lower)alkylglutamine, N<sup>6</sup>-trihalo(lower)-  
 alkoxycarbonyllysine, N<sup>6</sup>-ar(lower)alkoxycarbonyllysine, N<sup>7</sup>-arenesulfonylhistidine, N<sup>5</sup>-ar(lower)-  
 alkoxycarbonylornithine, R<sup>6</sup>-haloar(lower)alkoxycarbonyllysine, O<sup>3</sup>-ar(lower)alkylthreonine, N-lower alkylth-  
 reonine, O<sup>5</sup>-trihalo(lower)alkyl glutamate, O<sup>3</sup>-carboxy(lower)alkanoylthreonine, O<sup>3</sup>-glycylthreonine, O<sup>3</sup>-β-al-  
 anylthreonine, O<sup>3</sup>-(N-lower alkoxycarbonylglycyl)threonine and O<sup>3</sup>-(N-lower alkoxycarbonyl-β-alanyl)-  
 threonine.

6. A compound of claim 5, wherein

R<sup>3</sup> is a group of the formula :



wherein R<sup>4</sup> is hydrogen, lower alkyl, hydroxy(lower)alkyl or ar(lower)alkoxycarbonyloxy(lower)alkyl, and

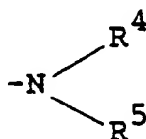
R<sup>5</sup> is aryl, ar(lower)alkyl or haloar(lower)alkyl.

7. A compound of claim 6, wherein

R<sup>1</sup> is hydrogen, t-butoxycarbonyl, formyl, benzyloxycarbonyl, acetyl, succinamoyl, t-butoxalyl, 3-diethylaminopropionyl, diethylaminoacetyl, 2-benzyl-t-butoxycarbonylaminoacetyl, (1H-tetrazol-1-yl)acetyl, 5-carboxyvaleryl, 4-carboxybutyryl, 3-carboxypropionyl, 4-morpholinecarbonyl, t-butylcarbonyl, (2-formamidothiazol-4-yl)acetyl, oxalo, carboxymethylaminoacetyl, benzylaminoacetyl or N-t-butoxycarbonyl-N-t-butoxycarbonylmethylaminoacetyl,

R<sup>2</sup> is hydrogen, formyl, tosyl, carbamoylmethyl, carboxymethyl or ethoxycarbonylmethyl, and

R<sup>3</sup> is a group of the formula :

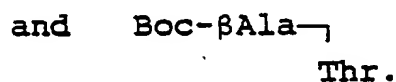
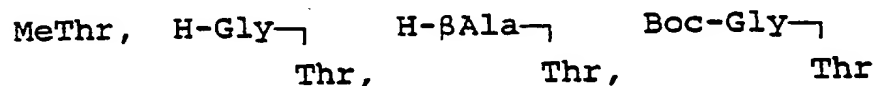
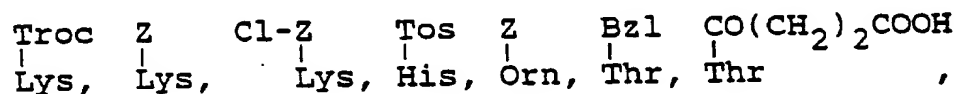
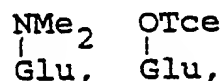


wherein R<sup>4</sup> is hydrogen, methyl, ethyl, hydroxyethyl or benzyloxycarbonyloxyethyl, and R<sup>5</sup> is phenyl, benzyl or O-fluorobenzyl.

8. A compound of claim 7, wherein

A is one amino acid residue selected from

Gln, Ser, Asn, Thr, D-Gln, Lys, His, βAsp, Orn, Gly, Tyr, D-Trp, Hyp, pGlu, Glu,



9. A compound of claim 8, which is selected from the group consisting of :

Boc-Gln-D-Trp(CHO)-Phe-NMeBzl,  
Boc-Thr-D-Trp(CHO)-Phe-NMeBzl,

Boc-Glu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-NMeBzl,  
 Ac-Thr-D-Trp(CHO)-Phe-NMeBzl, and  
 Ac-Glu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-NMeBzl.

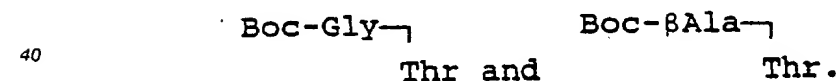
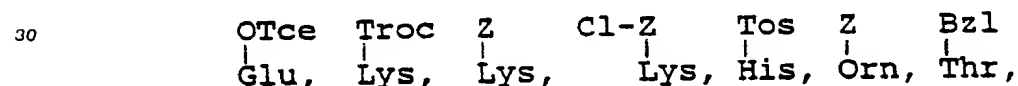
10. A compound of claim 5, wherein  
 5 R<sup>3</sup> is a group of the formula :  
 -OR<sup>6</sup>

wherein R<sup>6</sup> is aryl, lower alkyl, ar(lower)alkyl, haloar(lower)alkyl or pyridyl(lower)alkyl.

11. A compound of claim 10, wherein  
 10 R<sup>1</sup> is hydrogen, t-butoxycarbonyl, formyl, benzyloxy-carbonyl, acetyl, succinamoyl, t-butoxalyl, 3-diethylaminopropionyl, diethylaminoacetyl, 2-benzyl-t-butoxycarbonylaminoacetyl, (1H-tetrazol-1-yl)acetyl, 5-carboxyvaleryl, 4-carboxybutyryl, 3-carboxypropionyl, 4-morpholinecarbonyl, t-butylcarbamoyl, (2-formamidothiazol-4-yl)acetyl, oxalo, carboxymethylaminoacetyl, benzylaminoacetyl or N-t-butoxycarbonyl-N-t-butoxycarbonylmethylaminoacetyl,  
 15 R<sup>2</sup> is hydrogen, formyl, tosyl, carbamoylmethyl, carboxymethyl or ethoxycarbonylmethyl, and  
 R<sup>3</sup> is a group of the formula :  
 -OR<sup>6</sup>

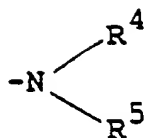
wherein R<sup>6</sup> is phenyl, methyl, isopropyl, benzyl, phenethyl, p-chlorobenzyl, 2-pyridylmethyl, 3-pyridylmethyl or 4-pyridylmethyl.

12. A compound of claim 11, wherein  
 20 A is one amino acid residue selected from Gln, Ser, Asn, Thr, D-Gln, Lys, His, βAsp, Orn, Gly, Tyr, D-Trp, Hyp, pGlu, Glu,



13. A compound of claim 12, which is selected from the group consisting of :  
 45 Boc-Gln-D-Trp(CHO)-Phe-OBzl,  
 Ac-Gln-D-Trp(CHO)-Phe-OBzl,  
 Z-Gln-D-Trp(CHO)-Phe-OBzl,  
 Boc-Asn-D-Trp(CHO)-Phe-OBzl,  
 Boc-Ser-D-Trp(CHO)-Phe-OBzl,  
 50 Boc-Glu(NMe<sub>2</sub>)-D-Trp(CHO)-Phe-OBzl, and  
 Boc-Thr-D-Trp(CHO)-Phe-OBzl.

14. A process for preparing a compound of the formula :  
 R<sup>1</sup>-A-D-Trp(R<sup>2</sup>)-Phe-R<sup>3</sup>  
 wherein R<sup>1</sup> is hydrogen or an amino protective group,  
 55 R<sup>2</sup> is hydrogen, an amino protective group, carbamoyl(lower)alkyl, carboxy(lower)alkyl or protected carboxy-(lower)alkyl,  
 R<sup>3</sup> is ar(lower)alkyl,  
 a group of the formula:



5

wherein  $\text{R}^4$  and  $\text{R}^5$  are each hydrogen, aryl or lower alkyl which may have suitable substituent(s),  
or

10  $\text{R}^4$  and  $\text{R}^5$  are linked together to form benzene-condensed lower alkylene, or

-OR<sup>6</sup>

wherein  $\text{R}^6$  is hydrogen, aryl or lower alkyl which may have suitable substituent(s), and

A is a single bond or one or two amino acid(s) residue, provided that when A is one amino acid residue of  
-D-Trp-, then  $\text{R}^4$  is not hydrogen,

15 or a pharmaceutically acceptable salt thereof,

which comprises,

(1) reacting a compound of the formula :

$\text{R}_3^1$ -A-D-Trp( $\text{R}^2$ )-OH

wherein  $\text{R}^2$  and A are each as defined above, and

20  $\text{R}_3^1$  is an amino protective group,

or its reactive derivative at the carboxy group or a salt thereof, with a compound of the formula :

H-Phe- $\text{R}^3$

wherein  $\text{R}^3$  is as defined above,

or its reactive derivative at the amino group or a salt thereof, to give a compound of the formula :

25  $\text{R}_3^1$ -A-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}_3^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and A are each as defined above,

or a salt thereof, or

(2) subjecting a compound of the formula :

$\text{R}_3^1$ -A-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

30 wherein  $\text{R}_3^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ , and A are each as defined above,

or a salt thereof, to elimination reaction of the amino protective group, to give a compound of the formula :

H-A-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}^2$ ,  $\text{R}^3$  and A are each as defined above, or a salt thereof, or

(3) reacting a compound of the formula :

35 H-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}^2$  and  $\text{R}^3$  are each as defined above, or its reactive derivative at the amino group or a salt thereof,  
with a compound of the formula:

$\text{R}_3^1$ -A'-OH

wherein  $\text{R}_3^1$ -A' is as defined above, and

40 A' is one or two amino acid(s) residue, or its reactive derivative at the carboxy group or a salt thereof, to  
give a compound of the formula :

$\text{R}_3^1$ -A'-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}_3^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and A' are each as defined above,

or a salt thereof, or

(4) subjecting a compound of the formula :

45 H-A-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}^2$ ,  $\text{R}^3$  and A are each as defined above, or its reactive derivative at the amino group or a salt  
thereof, to introduction reaction of the amino protective group, to give a compound of the formula :

$\text{R}_3^1$ -A-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

50 wherein  $\text{R}_3^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and A are each as defined above,

or a salt thereof, or

(5) reacting a compound of the formula :

H-A<sup>2</sup>-D-Trp( $\text{R}^2$ )-Phe- $\text{R}^3$

wherein  $\text{R}^2$  and  $\text{R}^3$  are each as defined above, and

55 A<sup>2</sup> is an amino acid residue,

or its reactive derivative at the amino group or a salt thereof, with a compound of the formula :

$\text{R}_3^1$ -A<sup>3</sup>-OH

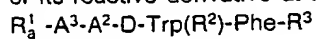
wherein



$R_a^1$  is as defined above, and

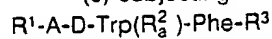
$A^3$  is an amino acid residue,

or its reactive derivative at the carboxy group or a salt thereof, to give a compound of the formula :



5 wherein  $R_a^1$ ,  $R^2$ ,  $R^3$ ,  $A^2$  and  $A^3$  are each as defined above, or a salt thereof, or

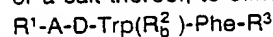
(6) subjecting a compound of the formula :



wherein  $R^1$ ,  $R^3$ , and  $A$  are each as defined above, and

$R_a^2$  is protected carboxy(lower)alkyl,

10 or a salt thereof, to elimination reaction of the carboxy protective group, to give a compound of the formula :



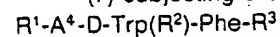
wherein

$R^1$ ,  $R^3$  and  $A$  are each as defined above, and

$R_a^2$  is carboxy(lower)alkyl,

15 or a salt thereof, or

(7) subjecting a compound of the formula :

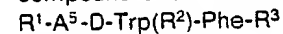


wherein  $R^1$ ,  $R^2$  and  $R^3$  are each as defined above, and

$A^4$  is one or two amino acid(s) residue containing a protected hydroxy group, a protected amino group or

20 protected imino group,

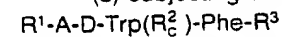
or a salt thereof, to elimination reaction of the amino, hydroxy or carboxy protective group, to give a compound of the formula :



wherein  $R^1$ ,  $R^2$  and  $R^3$  are each as defined above, and

25  $A^5$  is one or two amino acids residue containing a hydroxy group, an amino group or an imino group, or a salt thereof.

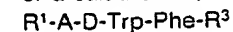
(8) subjecting a compound of the formula :



wherein  $R^1$ ,  $R^3$  and  $A$  are each as defined above, and

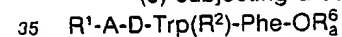
30  $R_c^2$  is an amino protective group,

or a salt thereof, to elimination reaction of the amino protective group, to give a compound of the formula :



wherein  $R^1$ ,  $R^3$  and  $A$  are each as defined above, or a salt thereof, or

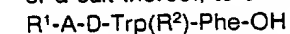
(9) subjecting a compound of the formula :



wherein  $R^1$ ,  $R^2$  and  $A$  are each as defined above, and

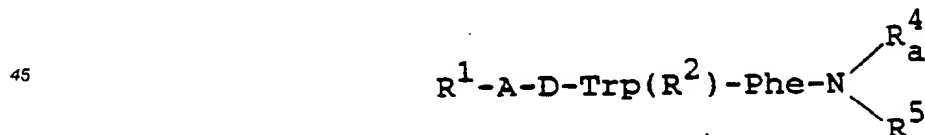
$R_a^6$  is lower alkyl which may have suitable substituent(s),

or a salt thereof, to elimination reaction of  $R_a^6$ , to give a compound of the formula :



40 wherein  $R^1$ ,  $R^2$  and  $A$  are each as defined above, or a salt thereof, or

(10) subjecting a compound of the formula :

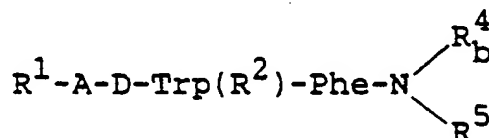


50 wherein  $R^1$ ,  $R^2$ ,  $R^5$  and  $A$  are each as defined above, and

$R_a^4$  is protected hydroxy(lower)alkyl,

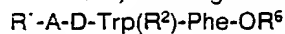
or a salt thereof, to elimination reaction of the hydroxy protective group, to give a compound of the formula

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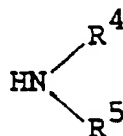


wherein  $R^1$ ,  $R^2$ ,  $R^5$  and A are each as defined above, and  
 $R^4$  is hydroxy(lower)alkyl,  
 or a salt thereof, or

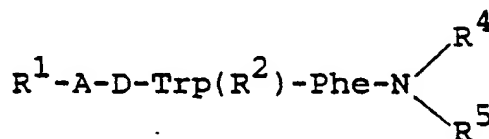
(11) reacting a compound of the formula :



wherein  $R^1$ ,  $R^2$ ,  $R^5$  and A are each as defined above,  
 or a salt thereof, with a compound of the formula:



wherein  $R^4$  and  $R^5$  are each as defined above, to give a compound of the formula :



wherein  $R^1$ ,  $R^2$ ,  $R^4$ ,  $R^5$  and A are each as defined above,  
 or a salt thereof, or

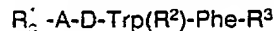
(12) subjecting a compound of the formula :  $R_6^1-A-D-Trp(R^2)-Phe-R^3$

wherein

$R^2$ ,  $R^3$  and A are each as defined above, and

$R_6^1$  is an amino protective group containing a protected carboxy,

or a salt thereof, to elimination reaction of the carboxy protective group, to give a compound of the formula :



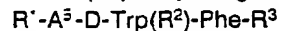
wherein

$R^2$ ,  $R^3$  and A are each as defined above, and

$R_6^1$  is an amino protective group containing a carboxy,

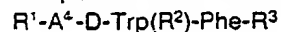
or a salt thereof, or

(13) subjecting a compound of the formula :



wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $A^5$  are each as defined above,

or a salt thereof, to introduction reaction of the amino, hydroxy or carboxy protective group, to give a compound of the formula :



wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $A^4$  are each as defined above,

or a salt thereof, or

(14) subjecting a compound of the formula :

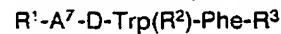


wherein

$R^1$ ,  $R^2$  and  $R^3$  are each as defined above, and

$A^5$  is one or two amino acid(s) residue which is substituted by acyl having protected amino,

or a salt thereof, to elimination reaction of the amino protective group, to give a compound of the formula :



wherein

$R^1$ ,  $R^2$  and  $R^3$  are each as defined above, and  
 $A^7$  is one or two amino acid(s) residue which is substituted by acyl having amino,  
 or a salt thereof, or

(15) subjecting a compound of the formula :

5  $R_d^1$ -A-D-Trp( $R^2$ )-Phe- $R^3$

wherein

$R^2$ ,  $R^3$  and A are each as defined above, and

$R_d^1$  is an amino protective group containing an amino group which is substituted by an amino protective  
 group and additionally a protected carboxy(lower)alkyl or an ar(lower)alkyl,

10 or a salt thereof, to elimination reaction of the amino and/or carboxy protective group, to give a compound  
 of the formula :

$R_e^1$ -A-D-Trp( $R^2$ )-Phe- $R^3$

wherein

$R^2$ ,  $R^3$  and A are each as defined above, and

15  $R_e^1$  is an amino protective group containing an amino group which is substituted by a carboxy(lower)alkyl or  
 an ar(lower)alkyl,  
 or a salt thereof or

(16) subjecting a compound of the formula :

H-Gln-D-Trp( $R^2$ )-Phe- $R^3$

20 wherein  $R^2$  and  $R^3$  are each as defined above, or a salt thereof, to ring closure reaction, to give a compound  
 of the formula :

pGlu-D-Trp( $R^2$ )-Phe- $R^3$

wherein  $R^2$  and  $R^3$  are each as defined above,  
 or a salt thereof, or

25 (17) reacting a compound of the formula :

$R^1$ -A-D-Trp( $R_d^2$ )-Phe- $R^3$

wherein  $R^1$ ,  $R_d^2$ ,  $R^3$  and A are each as defined above, or a salt thereof, with ammonia, to give a compound  
 of the formula :

$R^1$ -A-D-Trp( $R_d^2$ )-Phe- $R^3$

30 wherein

$R^1$ ,  $R^3$  and A are each as defined above, and

$R_d^2$  is carbamoyl(lower)alkyl,

or a salt thereof.

35 15. A pharmaceutical composition which comprises, as an active ingredient, a compound of claim 1 or a  
 pharmaceutically acceptable salt thereof in admixture with pharmaceutically acceptable carriers.

16. A use of a compound of claim 1 as a medicament.

17. A use of a compound of claim 1 as a tachykinin antagonist.

40 18. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for the manufacture of  
 a medicament for therapeutic treatment of asthma.

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(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 333 174 A3**

(12)

## EUROPEAN PATENT APPLICATION

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(51) Int. Cl.<sup>5</sup>: C07K 5/00, A61K 37/02

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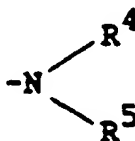
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(54) Peptide compounds, processes for preparation thereof and pharmaceutical composition comprising the same.

(57) A compound of the formula :  
R<sup>1</sup>-A-D-Trp(R<sup>2</sup>)-Phe-R<sup>3</sup>  
wherein

R<sup>1</sup> is hydrogen or an amino protective group,  
R<sup>2</sup> is hydrogen, an amino protective group,  
carbamoyl(lower)alkyl, carboxy(lower)alkyl or protect-  
ed carboxy(lower)alkyl,  
R<sup>3</sup> is ar(lower)alkyl,  
a group of the formula:



wherein R<sup>4</sup> and R<sup>5</sup> are each hydrogen, aryl or  
lower alkyl which may have suitable substituent-  
(s), or  
R<sup>4</sup> and R<sup>5</sup> are linked together to form benzene-

condensed lower alkylene, or  
a group of the formula :  
-OR<sup>6</sup>

wherein R<sup>6</sup> is hydrogen, aryl or lower alkyl  
which may have suitable substituent(s), and  
A is a single bond or one or two amino acid(s)  
residue, provided that when A is one amino acid  
residue of -D-Trp-, then R<sup>4</sup> is not hydrogen,  
and a pharmaceutically acceptable salt thereof,  
processes for its preparation and pharmaceutical  
compositions comprising them or a pharmaceutically  
acceptable salt thereof in admixture with phar-  
maceutically acceptable carriers.

EP 0 333 174 A3



European Patent  
Office

**PARTIAL EUROPEAN SEARCH REPORT**  
which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

Application number

EP 89 10 4617

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	US-A-4 223 020 (MOMANY)  * Column 4 (table 1; preparation); claims *	1-5, 10-11, 14-15	C 07 K 5/00 A 61 K 37/02
X	LIFE SCIENCE, vol. 31, 1982, pages 2249-2252, Pergamon Press Ltd, New York, US; R.J. VAVREK et al.: "Selectivity of minimum structure enkepholins"  * Page 2249 (Summary); page 2250, (Comp. 27) *	1,14-15	
X	DIPEPTIDES AND AMINO ACIDS, vol. 2, 1983, pages 369-370, George Thieme Verlag, Stuttgart, DE;  * Pages 369-370 (Item 1188) *	1,14	TECHNICAL FIELDS SEARCHED (Int. Cl.4)  C 07 K A 61 K
<b>INCOMPLETE SEARCH</b>  The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims. Claims searched completely: Claims searched incompletely: 16-17 Claims not searched: Reason for the limitation of the search:  Method for treatment of the human or animal body by surgery or therapy (See art. 52(4) of the European Patent Convention)			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>15-02-1991</b>	Examiner <b>KORSNER</b>
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document  T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons  & : member of the same patent family, corresponding document			

# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. **PCT/EP 98/00599**

International Filing Date **04 FEB 1998** (**04.02.98**)

EUROPEAN PATENT OFFICE  
PCT INTERNATIONAL APPLICATION  
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum) **1011PTWO**

**Box No. I TITLE OF INVENTION MONOCYCLIC COMPOUNDS WITH FOUR BIFUNCTIONAL RESIDUES HAVING NK-2 ANTAGONIST ACTION**

**Box No. II APPLICANT**

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (i.e. country) of nationality:  
ITALY

State (i.e. country) of residence:  
ITALY

This person is applicant  
for the purposes of:

☐ all designated  
States

☒ all designated States except  
the United States of America

☐ the United States  
of America only

☐ the States indicated in  
the Supplemental Box

**Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

GIORGI Raffaello  
Via delle Piagge 9  
56124 PISA - ITALY

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box  
is marked, do not fill in below.)

State (i.e. country) of nationality:  
ITALY

State (i.e. country) of residence:  
ITALY

This person is applicant  
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☐ all designated  
States

☐ all designated States except  
the United States of America

☒ the United States  
of America only

☐ the States indicated in  
the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

**Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The person identified below is hereby/has been appointed to act on behalf  
of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

PASSINI Angelo  
NOTARBARTOLO & GERVASI S.p.A.  
Corso di Porta Vittoria 9  
20122 MILAN - ITALY

Telephone No.

02/541799.1

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02/54179920

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.





Continuation of Box No. III FURTHER APPLICANTS, AND/OR (FURTHER) INVENTORS	
<i>If none of the following sub-boxes is used, this sheet is not to be included in the request.</i>	
<p><small>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)</small></p> <p>DI BUGNO Cristina Via R. Sanzio 16 56122 PISA - ITALY</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (i.e. country) of nationality: ITALY	State (i.e. country) of residence: ITALY
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><small>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)</small></p> <p>GIANNOTTI Danilo Via Roma 128 55011 ALTOPASCIO (Province of LUCCA) ITALY</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (i.e. country) of nationality: ITALY	State (i.e. country) of residence: ITALY
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><small>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)</small></p> <p>MAGGI Carlo Alberto Via Michelazzi 43 50141 FIRENZE - ITALY</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (i.e. country) of nationality: ITALY	State (i.e. country) of residence: ITALY
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><small>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)</small></p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (i.e. country) of nationality:	State (i.e. country) of residence:
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><input type="checkbox"/> Further applicants and/or (further) inventors are indicated on another continuation sheet.</p>	



**Box No.V DESIGNATION OF STATES**

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

**Regional Patent**

- ☒ **AP** ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

**National Patent** (if other kind of protection or treatment desired, specify on dotted line):

- |  |  |
|--|--|
| <input checked="" type="checkbox"/> AL Albania                               | <input checked="" type="checkbox"/> LT Lithuania                                 |
| <input checked="" type="checkbox"/> AM Armenia                               | <input checked="" type="checkbox"/> LU Luxembourg                                |
| <input checked="" type="checkbox"/> AT Austria                               | <input checked="" type="checkbox"/> LV Latvia                                    |
| <input checked="" type="checkbox"/> AU Australia                             | <input checked="" type="checkbox"/> MD Republic of Moldova                       |
| <input checked="" type="checkbox"/> AZ Azerbaijan                            | <input checked="" type="checkbox"/> MG Madagascar                                |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina                | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BB Barbados                              |  |
| <input checked="" type="checkbox"/> BG Bulgaria                              | <input checked="" type="checkbox"/> MN Mongolia                                  |
| <input checked="" type="checkbox"/> BR Brazil                                | <input checked="" type="checkbox"/> MW Malawi                                    |
| <input checked="" type="checkbox"/> BY Belarus                               | <input checked="" type="checkbox"/> MX Mexico                                    |
| <input checked="" type="checkbox"/> CA Canada                                | <input checked="" type="checkbox"/> NO Norway                                    |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> NZ New Zealand                               |
| <input checked="" type="checkbox"/> CN China                                 | <input checked="" type="checkbox"/> PL Poland                                    |
| <input checked="" type="checkbox"/> CU Cuba                                  | <input checked="" type="checkbox"/> PT Portugal                                  |
| <input checked="" type="checkbox"/> CZ Czech Republic                        | <input checked="" type="checkbox"/> RO Romania                                   |
| <input checked="" type="checkbox"/> DE Germany                               | <input checked="" type="checkbox"/> RU Russian Federation                        |
| <input checked="" type="checkbox"/> DK Denmark                               | <input checked="" type="checkbox"/> SD Sudan                                     |
| <input checked="" type="checkbox"/> EE Estonia                               | <input checked="" type="checkbox"/> SE Sweden                                    |
| <input checked="" type="checkbox"/> ES Spain                                 | <input checked="" type="checkbox"/> SG Singapore                                 |
| <input checked="" type="checkbox"/> FI Finland                               | <input checked="" type="checkbox"/> SI Slovenia                                  |
| <input checked="" type="checkbox"/> GB United Kingdom                        | <input checked="" type="checkbox"/> SK Slovakia                                  |
| <input checked="" type="checkbox"/> GE Georgia                               | <input checked="" type="checkbox"/> SL Sierra Leone                              |
| <input checked="" type="checkbox"/> GH Ghana                                 | <input checked="" type="checkbox"/> TJ Tajikistan                                |
| <input checked="" type="checkbox"/> GM Gambia                                | <input checked="" type="checkbox"/> TM Turkmenistan                              |
| <input checked="" type="checkbox"/> GW Guinea-Bissau                         | <input checked="" type="checkbox"/> TR Turkey                                    |
| <input checked="" type="checkbox"/> HU Hungary                               | <input checked="" type="checkbox"/> TT Trinidad and Tobago                       |
| <input checked="" type="checkbox"/> ID Indonesia                             | <input checked="" type="checkbox"/> UA Ukraine                                   |
| <input checked="" type="checkbox"/> IL Israel                                | <input checked="" type="checkbox"/> UG Uganda                                    |
| <input checked="" type="checkbox"/> IS Iceland                               | <input checked="" type="checkbox"/> US United States of America                  |
| <input checked="" type="checkbox"/> JP Japan                                 |  |
| <input checked="" type="checkbox"/> KE Kenya                                 | <input checked="" type="checkbox"/> UZ Uzbekistan                                |
| <input checked="" type="checkbox"/> KG Kyrgyzstan                            | <input checked="" type="checkbox"/> VN Viet Nam                                  |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> YU Yugoslavia                                |
|  | <input checked="" type="checkbox"/> ZW Zimbabwe                                  |
| <input checked="" type="checkbox"/> KR Republic of Korea                     |  |
| <input checked="" type="checkbox"/> KZ Kazakhstan                            |  |
| <input checked="" type="checkbox"/> LC Saint Lucia                           |  |
| <input checked="" type="checkbox"/> LK Sri Lanka                             |  |
| <input checked="" type="checkbox"/> LR Liberia                               |  |
| <input checked="" type="checkbox"/> LS Lesotho                               |  |

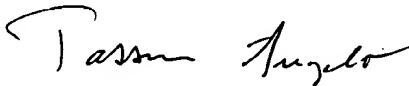
Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of .....

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)



<b>Box No. VI PRIORITY CLAIM</b>		Further priority claims are indicated in the Supplemental Box <input type="checkbox"/>	
The priority of the following earlier application(s) is hereby claimed:			
Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1)  ITALY	7th February 1997	FI97A000020	
item (2)			
item (3)			
Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required): <input type="checkbox"/> The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): _____			
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This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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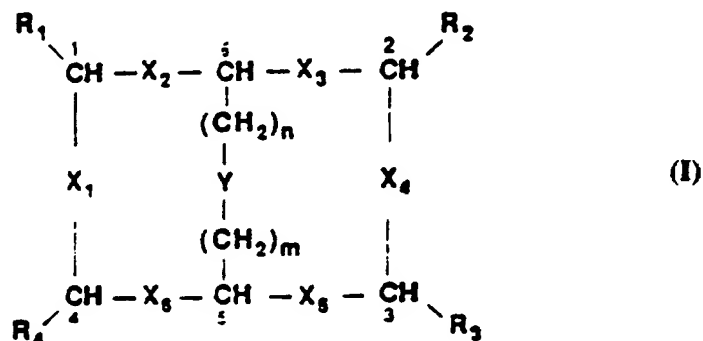
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/EP96/01028 <b>(22) International Filing Date:</b> 11 March 1996 (11.03.96) <b>(30) Priority Data:</b> FI95A000044      13 March 1995 (13.03.95)      IT <b>(71) Applicant (for all designated States except US):</b> A. MENARINI INDUSTRIE FARMACEUTICHE RIUNITE S.R.L. [IT/IT]; Via Sette Santi, 3, I-50131 Florence (IT). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ARCAMONE, Federico [IT/IT]; Via 4 Novembre, 26, I-20014 Nerviano (IT). MAGGI, Carlo, Alberto [IT/IT]; Via Michelazzi, 43, I- 50100 Florence (IT). QUARTARA, Laura [IT/IT]; Viale Os- imo, 385, I-52037 Sansepolcro (IT). GIANNOTTI, Danilo [IT/IT]; Via Roma, 128, I-55011 Altopascio (IT). <b>(74) Agent:</b> GERVASI, Gemma; Notarbartolo & Gervasi S.r.l., Viale Bianca Maria, 33, I-20122 Milan (IT).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>

**(54) Title:** BICYCLIC TACHYKININS ANTAGONISTS. PREPARATION THEREOF AND THEIR USE IN PHARMACEUTICAL COMPOSITION

**(57) Abstract**

This invention relates to novel compounds of general formula (I) and to pharmaceutical compositions containing them.



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BYCYCLIC TACHYKININS ANTAGONISTS, PREPARATION THEREOF AND  
THEIR USE IN PHARMACEUTICAL COMPOSITION

Field of the Invention

This invention relates to novel bi-cyclic compounds useful in  
pharmaceutical compositions as tachykinins antagonists, and to  
pharmaceutical compositions containing them.

Background of the invention

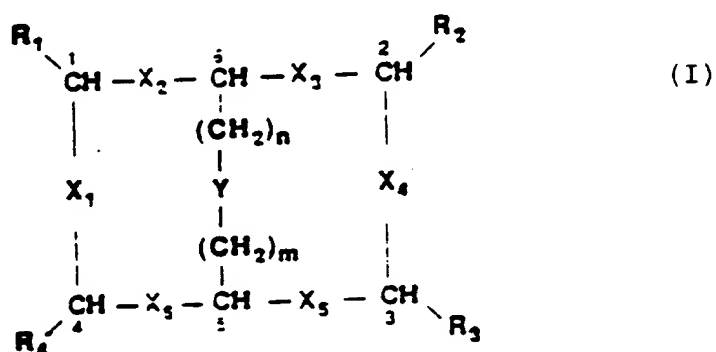
The receptor  $NK_2$  of tachykinins is widely expressed in the  
peripheral nervous system of Mammalia. One of the several effects  
caused by the selective stimulation of the receptor  $NK_2$  is the  
contraction of the smooth muscles. Therefore, antagonists of the  
receptor  $NK_2$  can be considered agents able to control the  
hypercontraction of the smooth muscles in any pathological condition in  
which the release of the tachykinins contributes to the rise of the  
corrispondent disorder. In particular, the bronchospastic component of  
asthma, cough, pulmonary irritations and local spasms of the urinary  
bladder and of the ureter during cystitis, infections and renal colics  
can be considered conditions in which the administration of receptor  
 $NK_2$  antagonists can be effective (A.L. Magnan et al. *Neuropeptides*,  
1993, 24, 199). Compounds which act as antagonists of the tachykinins,  
and in particular of the neurokinin A, are well-known in Literature.  
Among them, the cyclic compounds (B. J. Williams et al. *J. Med. Chem.*,  
1993, 36, 2) are of particular interest. Lipophily has been defined as  
an essential requirement in order to have an intensive antagonist  
activity to the receptor  $NK_2$  of the tachykinins of a series of cyclic  
pseudopeptides (L. Quartara et al. *J. Med. Chem.*, 1994, 27) and

particularly in case of bicyclic hexapeptides. W0/ 93/21227)). Surprisingly it has been now found that products structurally similar to those described above, but in which, however, at least one hydrophilic group is present, not only keep their high affinity *in vitro*, but also show an increase in the pharmacological activity *in vivo* if compared to the corrispondent compounds which do not contain any hydrophilic group.

This is even more surprising if it is taken into account that monocyclic peptides having antagonist properties which are similar to those of the tachykinins do not show any increase in the pharmacological activity when hydrophilic groups are introduced onto the structure of the cycle [Int. J. Peptide Protein Res. (1984), 44:2, 105-111].

## Summary

15 This invention relates to novel compounds of the general formula (I):



wherein:

$X_1, X_2, X_3, X_4, X_5$ , and  $X_6$ , same or different from one another,  
represent a -NR'CO- or a -CONR'- group, wherein R' is H or  $C_{1-3}$   
alkyl;

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Y represents a group selected from -NRCO-, -CONR-, or -SS-

wherein R is H or C<sub>1-3</sub> alkyl;

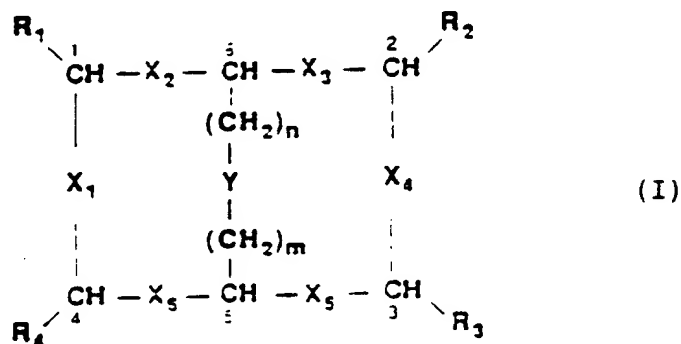
at least one of the R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> groups, same or different from one another, is hydrophilic and the remaining groups are hydrophobic;

5 m and n, same or different from one another, are each an integer number from 1 to 4;

and to pharmaceutical compositions containing them.

#### Detailed description of the Invention

The present invention relates to novel compounds having the general  
10 formula (I)



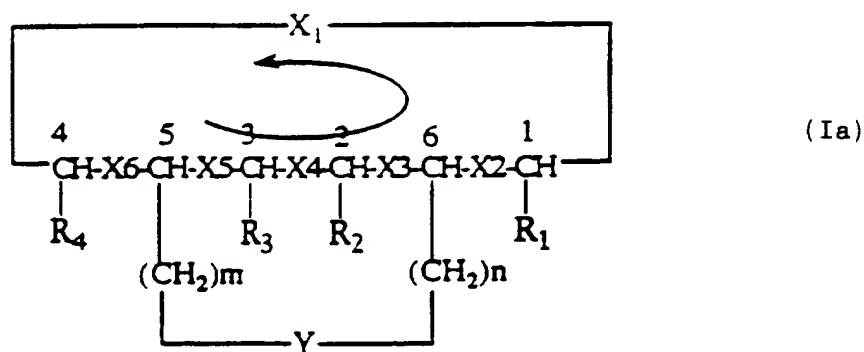
wherein

X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>; Y, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, m and n groups are as defined above;

processes for the preparation thereof and pharmaceutical compositions containing them.

The formula (I) as reported above is considered the one giving the  
15 best representation of the real spatial structure of the bicyclic peptide according to the invention. However also the following Formula (Ia) (which chemically speaking is identical to Formula (I)) is given

in order to simplify the understanding of the compounds described hereinafter and in the Examples with their chemical name in particular in so far as the groups  $X_{1-6}$  and Y are concerned.



The groups  $X_{1-6}$  and Y are in fact defined according to the aminoacid-sequence from the formal N- to the C-terminus of the peptide as they are represented in the linear structure, therefore reading Formula (Ia) no problem arises in the understanding of the linear structure as reported in the Examples.

As it can be seen, the compounds of formula (I) as described above present chiral centers: it is understood that this invention relates also to the several enantiomers.

More particularly the hydrophobic groups can be separately selected from the following:

- a) groups  $C_nH_{2n+1}$  wherein  $n = 0, 1-4$
- b) linear- or branched alkyl groups corresponding to  $C_nH_{2n}-U-W$  wherein  $n = 1-4$ ;  $U = O, COO, CONH, S$  and  $W = \text{alkyl-, aryl or alkylaryl-group}$  containing from 1 to 15 carbon atoms
- c)  $(CH_2)_n - C_6H_3 - A - B$  wherein  $n = 0, 1-3$ ; A and B, placed in any of the ortho, meta or para positions, same or different from one another, represent H, halogen, OR, NHR,  $NR_2$ ,  $CH_3$ , SR wherein R is an alkyl-, aryl- or alkylaryl-group with less than 10 C atoms
- d)  $(CH_2)_n - C_6H_{10} R'$  wherein  $n = 0, 1-3$  and  $R' = H, C_{1-3} \text{ alkyl}$



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e)  $(CH_2)_n$  -heterocycle, wherein  $n = 0, 1-3$  and for heterocycle it is meant: imidazolyl-2-yl, indolyl-3-yl, furanyl-3-yl, pyridyl-3-yl, imidazolyl-3-yl

f) a  $-(CH_2)_s-$  group, wherein  $s = 3, 4$ , eventually OH-substituted or  
5 condensed with an aromatic group, which cyclizes with one of the two adjacent  $X_{1-6}$  groups in order to produce the side chain of proline, hydroxyproline, octahydroindol-2-carboxylic acid, tetrahydroisoquinolinic acid

g) the side chain of a natural hydrophobic amino acid

10 h) the side chain of a natural hydrophilic amino acid, suitably substituted in order to render it hydrophobic

i) the side chain of non-natural hydrophobic amino acids selected from the group consisting of: norleucine, norvaline, alloisoleucine, cyclohexylglycine (Chg),  $\alpha$ -amino-n-butyric acid (Aba),  
15 cyclohexylalanine (Cha), aminophenylbutyric acid (Pba), phenylalanines mono- and di- substituted in the ortho, meta and para positions of the benzene ring with one or more of the following groups:  $C_{1-10}$  alkyl,  $C_{1-10}$  alkoxy, halogen,  $\beta$ -2-thienylalanine,  $\beta$ -3-thienylalanine,  $\beta$ -2-furanylalanine,  $\beta$ -3-furanylalanine,  $\beta$ -2-piridylalanine,  $\beta$ -3-piridylalanine,  $\beta$ -4-piridylalanine,  $\beta$ -(1-naphtyl)alanine,  $\beta$ -(2-naphtyl)alanine, O-alkylated serine- threonine- tyrosine-derivatives,  
20 S-alkyl cysteine, S-alkyl homocysteine, N-alkyl lysine, N-alkyl ornithine, N-alkyl 2,3 diaminopropionic acid.

More particularly, the side chain of a hydrophobic amino acid  
25 according to paragraph (g) is the side chain of an amino acid selected from the group consisting of: glycine, alanine, valine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan, proline, histidine,

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asparagine, glutamine.

The side chain of a hydrophilic amino acid, suitably substituted in order to render it hydrophobic according to paragraph (h) is the chain of an amino acid selected from the group consisting of: serine,

5 threonine, cysteine, aspartic acid, glutamic acid, t-carboxyglutamic acid, arginine, ornithine, lysine.

Preferably, the hydrophilic groups are selected from L-Q group, wherein L is a chemical bond or a linear or branched C<sub>1-6</sub>-alkyl residue and Q is a hydrophilic group. Preferably Q is selected from

10 the group consisting of: guanidine, amine, M, OM, -CO-NH-M, -NH-CO-M, an aromatic group which has been mono-, di- or tri-substituted in ortho, meta, para positions with M or OM groups, wherein M is a hydrophilic group.

With the term "hydrophilic group", for Q and M, it is preferably  
15 meant:

i) eventually substituted mono-, di-, tri-glycosidic residues:

ii) C<sub>1-6</sub> linear or cyclic alkyl chains comprising one or more polar groups;

iii) hydroxyl, amine, guanidine, carboxyl, sulfate, phosphonate,  
20 phosphate;

iv) residues bearing substituted hydrophilic groups which in biologic environment are hydrolysed, re-establishing the hydrophilic function.

As far as the definition according to paragraph (i) hereinabove is  
25 concerned, the following structures are preferably meant:

hexoses or pentoses of the D or L series in  $\alpha$  or  $\beta$  configuration, selected from the group wherein: all C atoms bear a free or protected

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hydroxylic group; one or more hydroxyls are substituted by: hydrogen, an amino or acylamino group; C<sub>6</sub> of hexoses and C<sub>5</sub> of pentoses are part of a carboxylic group; and wherein the eventually present 2 or 3 glycosidic units are linked by a glycosidic bond of  $\alpha$  or  $\beta$  configuration.

Specific examples of glycosidic groups as defined above are: D or L ribose, D or L arabinose, D or L xylose, D or L lyxose, D or L allose, D or L altrose, D or L glucose, D or L mannose, D or L gulose, D or L idose, D or L galactose, D or L talose, D or L allulose, D or L fructose, D or L sorbose, D or L tagatose; 5-deoxy-D or L-arabinose, 2-deoxy-D or L-glucose, 2-deoxy-D or L-galactose, 2-deoxy-D or L-arabinose, 2-deoxy-D or L-ribose, D or L fucose, D or L ramnose; D-glucosamine, D-mannosamine, D-galactosamine, daunosamine, acosamine and N-acylate derivatives thereof with lower fatty acids, i.e. having a N-formylic, acetylic, propionilic, butyric residue; glucuronic acid, galacturonic acid, cellobiose, lactose, maltose, D-lactosamine, cellotriose, maltotriose and protected derivatives thereof.

The definition according to paragraph (ii) hereinabove applies to chains deriving from a polyol-residue, such as tris(hydroxymethyl)methyl, D or L arabitol, D or L erythrol, D or L galactitol, meso-inositol, D or L mannitol, D or L perseitol, D or L ribitol, D or L sorbitol, D or L xylitol; or those deriving from the residue of tartaric acid, glucaric acid, gluconic acid, bycine, quinic acid, mucic acid, glucosaminic acid.

Among the products of formula (I) as above indicated, the products wherein if one or both R<sub>1</sub> and R<sub>4</sub> groups are hydrophilic, both R<sub>2</sub> and R<sub>3</sub> groups are hydrophobic and viceversa, are particularly preferred.

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Compounds of formula (I) object of the present invention can be synthesized by the various techniques known in Literature, see e.g. M. Bodansky, "Peptide Chemistry", Springer-Verlag, 1988.

For example by means of in solution synthesis of the linear peptidic chain through subsequent coupling of suitably activated N-protected amino acids to an amino acid or to a C-protected peptidic chain, with isolation of the intermediates, subsequent selective de-protection of the C- and N-terminal chains, cyclization in polar organic solvents in diluted solution, hence selective de-protection of the side chains and at last cyclization of the same in polar organic solvents in diluted solution. The hydrophilic residue can be introduced both as protected amino acid derivative during the peptidic chain synthesis and by means of conjugation to the already formed peptide, as widely disclosed in Literature. Similarly a synthesis in solid phase of the peptidic chain from the C-terminal end to the N-terminal one on a insoluble polymeric support, the cyclization in solid phase between the previously de-protected side chains, the subsequent detachment from the polymeric support by means of hydrolysis in anhydrous hydrofluoric acid containing the suitable scavengers or in trifluoroacetic acid containing the suitable scavengers or in aqueous bases, and the cyclization of the monocyclic peptide in polar organic solvents in diluted solution, can be used for the preparation. The hydrophilic residue being introduced according to the above disclosed indications. According to a particular preparation method, the desired product can be obtained in solid phase using the 2-chlorotrytil resin (Barlos et al., Int. J. Peptide Protein Res., 37, 513-520, 1991) substituted with a protected amino acid having the Fmoc group at the N-terminal end;

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preferably the amino acid directly bond to the resin is the one having the  $R_1$  or  $R_3$  side chain. After the other amino acids being introduced in the sequence, the peptide is detached from the resin with diluted acetic acid and a first cyclization is performed between the free C-terminal and N-terminal end by means of the conventional classic synthesis methods. Subsequently, the amino acid side chains are de-protected in position 5 and 6, for example with trifluoroacetic acid, and way is given to the second cyclization.

Other synthetic ways are anyway possible and largely described in Literature as above mentioned.

The compounds of formula (I) as above indicated have revealed to be powerful antagonists of the receptor  $NK_2$  of the tachykinins, and hence may be administered in doses which are not higher than those required for the known products.

They can be therefore indicated for the treatment of arthritis, asthma, inflammations, tumoral growth, gastro-intestinal hypermotility, Huntington's disease, neurites, neuralgia, hemicrania, hypertension, urinary incontinence, urticaria, symptoms from carcinoid disease, flu and colds.

The compounds of formula (I) object of the present invention are suitable for the parenteral, oral, inhalatory and sublingual administration for therapeutical purposes to the superior animals and to the humans, achieving pharmacological effects according to the above described features. For parenteral administrations (endovenous, intramuscular and intradermic) sterile solutions or lyophilized chemical preparations are used. For nasal, inhalatory and sublingual administrations, according to the particular instance,

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aqueous solutions, aerosol preparations or capsules are used.

The doses of active principle in the above compositions can be comprised between 0.1 and 10 mg/kg of body weight.

#### EXAMPLE 1.

5 Preparation of cyclo([Asn( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 1) compound of formula (I) wherein  $Y=X_1=X_2=X_3=X_4=X_5=X_6=-$ CO-NH-;  $R_1=-CH_2-CH(CH_3)_2$ ;  $R_2=-CH_2-C_6H_5$ ,  $R_3=-CH_2$ indolyl-3-yl,  $R_4=-CH_2-CO-NH-(\beta$ -D-Glc);  $m=n=1$  and the carbon atoms  $C_1, C_2, C_3, C_4, C_5, C_6$  have L configuration].

10 a) synthesis of the linear peptide H-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-Asp(OtBu)-Trp-Phe-Dap(Boc)-Leu-OH.

1 g of 2-chlor trityl resin (1.6 mmol/g, Novabiochem) is functionalized with Fmoc-Leu-OH (0.6 eqs.) as described by Barlos et al., Int. J. Peptide Protein Res., 1991, 37, 513-520. The substitution  
15 degree of the resin is determined by dosing the group Fmoc, and it is equal to 0.364 meq/g. The subsequent 4 amino acids are coupled as free acids using an excess 3 of amino acid and HOBt (4 eqs.) and DCC (3 eqs.) as activators with reaction times of 1 hour. In the following order: Fmoc-Dap(Boc)-OH, Fmoc-Phe-OH, Fmoc-Trp-OH, Fmoc-Asp(OtBu)-OH  
20 are added. The last amino acid is coupled as Fmoc-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp (Christiansen-Brams et al., J.Chem.Soc. Perkin Trans. I, 1993, 1461-1471), 2 eqs., with HOBt (2 eqs.) as activator, for 3h.

After the de-protection of the group Fmoc, the detachment from the resin is performed, suspending it in 10 mL of a mixture of AcOH, TFE,  
25 DCM (1/1/8, v/v) at room temperature for 0.5 h. Thereafter the solvent is evaporated under vacuum at 30°C, it is again mixed with water and it is lyophilized. Yield in raw product: 405 mg (90 %). Title HPLC: 70

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%. FAB-MS:  $[M+H]^+ = 1266$ ;  $t_R$ : 14.7 min.

b) Synthesis of the bicyclic product cyclo([Asn((Ac<sub>4</sub>O)-β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)) (compound 2).

The linear raw product is cyclized in 1 mM solution in DMF, at 4°C, with 1 eq. of PyBOP and 1.2 eqs. of DIEA for 1 h. The mixture is dried and purified in HPLC obtaining 156 mg of the pure product (yield 39 %). Title HPLC: >99 %. FAB-MS:  $[M+H]^+ = 1248$ ;  $t_R$ : 18.4 min.

The monocyclic product is de-protected by solving it in 15 ml of TFA containing water at 10 %. After 0.5 h. the mixture is diluted in water and it is lyophilized. The residue is dissolved in 1 mM solution in DMF, the solution is brought to 0°C and 1 eq. of PyBOP and 1.2 eqs. of DIEA are added. After 5 h. it is dried and purified in HPLC. Yield 45 % (70 mg). Title HPLC > 99 %. FAB-MS:  $[M+H]^+ = 1074$ ;  $t_R$ : 13.5 min.

c) Synthesis of the bicyclic product cyclo ([Asn(β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β))

70 mg of tetraacetate product are dissolved in anhydrous methanol in 5 mM solution. The solution is brought to -20°C and a 1 mM solution of sodium methylate in methanol is added to achieve pH = 11. After 10' acetic acid is added to achieve neutral pH, high dilution with water and lyophilization follow. Yield 60 %. Title HPLC: 98 %. FAB-MS:  $[M+H]^+ = 906$ ;  $t_R$ : 9.3 min.

#### EXAMPLE 2

Preparation of cyclo([Ser(β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)) (SEQ ID No. 2) [compound of Formula (I) wherein:  $Y = X_1 = X_2 = X_3 = X_4 = X_5 = X_6 =$  CO-NH-;  $R_1 = -CH_2-CH(CH_3)_2$ ;  $R_2 = -CH_2-C_6H_5$ ;  $R_3 = -CH_2$ -indolyl-3-yl;  $R_4 = -CH_2-O-(\beta-D-Glc)$ ;  $m = n = 1$  and  $C_1, C_2, C_3, C_4, C_5, C_6$  carbon atoms have L configuration].

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a) synthesis of linear peptide H-Ser[(Bz<sub>4</sub>O)-β-D-Glc]-Asp(OtBu)-Trp-Phe-Dap(Boc)-Leu-OH.

The same procedure which has been used for Example 1), paragraph a), is utilized here till the addition of the last amino acid, which is  
5 coupled as Fmoc-Ser[(Bz<sub>4</sub>O)-β-D-Glc]-OPfp (obtained by the procedure which has been described by Vargas-Berenguel et al., J. Chem. Soc. Perkin Trans. I, 1994, 2615, 2619).

The detachment occurs as described above, in Example 1). Yield in raw  
product: 450 mg (83 %). Title HPLC: 93 %. FAB-MS: [M+H]<sup>+</sup> = 1487; t<sub>R</sub>:  
10 20.8 min.

b) Synthesis of bicyclic product cyclo([Ser[(Bz<sub>4</sub>O)-β-D-Glc]-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)).

The linear raw product is cyclized in 1mM solution in DMF, at 4°C, with 1 eq. of PyBOP and 1.2 eqs. of DIEA for 1 h. The mixture is dried  
15 and purified in HPLC, obtaining 0.16 g of pure product (yield 35 %). Title HPLC: >99 %. FAB-MS: [M+H]<sup>+</sup> = 1469; t<sub>R</sub>: 25.3 min.

The monocyclic product is de-protected by liquefying it in 10 mL of TFA containing water at 10 %. After 0.5 h the mixture is diluted in water and it is lyophilized. The residue is dissolved in 1mM solution  
20 in DMF, the solution is brought to 0°C and 1 eq. of PyBOP and 1.2 eqs. of DIEA are added. After 24 h it is dried and purified in HPLC. Yield 63 mg (45 %). Title HPLC: >99 %. FAB-MS: [M+H]<sup>+</sup> = 1295; t<sub>R</sub>: 21.6 min.

c) Synthesis of bicyclic product cyclo([Ser(β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)).

25 20 mg of tetrabenzoylate product are dissolved in anhydrous methanol in 5mM solution. The solution is brought to -20°C and a 1mM solution of sodium methylate in methanol is added to achieve pH = 11. After 1.5



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n acetic acid is added to achieve neutral pH, high dilution with water and lyophilization follow. Yield: 6.5 mg (48 %). Title HPLC: > 99 %. FAB-MS:  $[M+H]^+ = 878$ ;  $t_R$ : 9.6 min.

By similar procedures, the following compounds have been obtained:

5 EXAMPLE 3

cyclo([Asn( $\beta$ -D-2-deoxy-2-amino-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 3) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-amino-Glc) and the other substituents are as defined in Example 1].

10 EXAMPLE 4

cyclo ([Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 4) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-acetamido-Glc) and the other substituents are as defined in Example 1].

15 EXAMPLE 5

cyclo ([Nle-Asp-Trp-Phe-Dap-Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 5) [compound of Formula I) wherein  $R_1 = -CH_2-CO-NH-(\beta$ -D-2-deoxy-2-acetamido-Glc),  $R_4 = -(CH_2)_3-CH_3$ ] and the other substituents are as defined in Example 1].

20 EXAMPLE 6

cyclo([Asn( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 6) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta$ -D-ribofuranosyl) and the other substituents are as defined in Example 1].

25 EXAMPLE 7

cyclo([Ser( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 7) [compound of Formula I) wherein  $R_4 = -CH_2-O-(\beta$ -D-

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ribofuranosyl). and the other substituents are as defined in Example 1].

## EXAMPLE 8

cyclo ([Asn ( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
5 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 8) [compound of Formula I) wherein  $R_4$ = -CH<sub>2</sub>-CO-  
NH-( $\beta$ -L-arabinofuranosyl) and the other substituents are as defined in  
Example 1].

## EXAMPLE 9

cyclo ([Ser ( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
10 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 9) [compound of Formula I) wherein  $R_4$ = -CH<sub>2</sub>-O-( $\beta$ -  
L-arabinofuranosyl) and the other substituents are as defined in  
Example 1].

## EXAMPLE 10

cyclo([Asn( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
15 (SEQ ID 10) [compound of Formula I) wherein  $R_4$ = -CH<sub>2</sub>-CO-NH-( $\beta$ -D-  
mannopyranosyl) and the other substituents are as defined in Example  
1].

## EXAMPLE 11

cyclo([Ser( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
20 (SEQ ID No. 11) [compound of Formula I) wherein:  $R_4$ = -CH<sub>2</sub>-O-( $\beta$ -D-  
mannopyranosyl) and the other substituents are as defined in Example  
1].

## EXAMPLE 12

cyclo ([Asn ( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
25 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 12) [compound of Formula I) wherein  $R_4$ = -CH<sub>2</sub>-CO-  
NH-( $\beta$ -D-galactopyranosyl) and the other substituents are as defined in  
Example 1].

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## EXAMPLE 13

cyclo([Ser( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )  
(SEQ ID No. 13) [compound of Formula I) wherein  $R_4 = -CH_2-O-(\beta$ -D-galactopyranosyl) and the other substituents are as defined in Example  
5 1].

## EXAMPLE 14

cyclo ([Asn( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 14) [compound of Formula I) wherein  $R_4 = -CH_2-CO-$   
NH-( $\beta$ -D-glucuronopyranosyl) and the other substituents are as defined  
10 in Example 1].

## EXAMPLE 15

cyclo ([Ser( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 15) [compound of Formula I) wherein  $R_4 = -CH_2-O-$   
( $\beta$ -D-glucuronopyranosyl) and the other substituents are as defined in  
15 Example 1].

## EXAMPLE 16

cyclo ([Asn(1-deoxy-sorbitol-1-yl)-Asp-Trp-Phe-Dap-Leu] cyclo  
(2 $\beta$ -5 $\beta$ )) (SEQ ID 16) [compound of Formula I) wherein  $R_4 = -CH_2-CO-NH-$   
(1-deoxy-sorbitol-1-yl) and the other substituents are as defined in  
20 Example 1].

## EXAMPLE 17

cyclo ([Asn[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc]]-Asp-Trp-Phe-Dap-Leu]cyclo-  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 17) [compound of Formula I) wherein  $R_4 = -CH_2-CO-$   
NH-[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc]] and the other substituents are as defined  
25 in Example 1].

## EXAMPLE 18

cyclo([Asn[4-O-( $\alpha$ -D-galactopyranosyl)- $\beta$ -D-Glc]-Asp-Trp-Phe-Dap-

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Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 18) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-[4-O( $\beta$ -D-galactopyranosyl)- $\beta$ -D-Glc]] and the other substituents are as defined in Example 1].

## EXAMPLE 19

5 cyclo ([ Asn [ O- $\alpha$ -D-Glc-(1-4)-O- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc]-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 19) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-[O- $\alpha$ -D-Glc-(1-4)-O- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc) and the other substituents are as defined in Example 1].

## EXAMPLE 20

10 cyclo([Asn(D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 20) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-CO-NH-(D-2-deoxy-glucopyranos-2-yl) and the other substituents are as defined in Example 1].

## EXAMPLE 21

15 cyclo ([Dap[D(-)-quinyl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 21) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-NH-[D(-)-quinyl], and the other substituents are as defined in Example 1].

## EXAMPLE 22

cyclo ([Dap[D-gluconyl]-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))  
20 (SEQ ID No. 22) [compound of Formula I) wherein: R<sub>4</sub> = -CH<sub>2</sub>-NH-(D-gluconyl) and the other substituents are as defined in Example 1].

## EXAMPLE 23

cyclo ([Dap[D-glucuryl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
(SEQ ID No. 23) [compound of Formula I) wherein R<sub>4</sub> = -CH<sub>2</sub>-NH-(D-glucuryl) and the other substituents are as defined in Example 1].  
25

## EXAMPLE 24

cyclo ([Dap(2-sulfo-benzoyl)-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ ))

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(SEQ ID No. 24) [compound of Formula I) wherein:  $R_4 = -CH_2-NH-CO-C_6H_4-SO_3H$  and the other substituents are as defined in Example 1].

## EXAMPLE 25

cyclo ([Asn (4-sulfo-phenyl)-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ ))

5 (SEQ ID No. 25) [compound of Formula I) wherein  $R_4 = CH_2-CO-NH-C_6H_4-SO_3H$  and the other substituents are as defined in Example 1].

## EXAMPLE 26

cyclo([Asn( $\beta$ -L-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 26)

[compound of Formula I) wherein  $R_4 = -CH_2-CO-NH(\beta$ -L-Glc) and the other  
10 substituents are as defined in Example 1].

## EXAMPLE 27

cyclo([Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-

Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 27) [compound of formula I) wherein  $R_4$   
=  $-CH_2-CO-NH-(D-2-deoxy-glucopyranos-2-yl)$  and the other substituents  
15 are as defined in Example 1].

## EXAMPLE 28

cyclo ([Asn(D-2-deoxy-mannopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]-

cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 28) [compound of formula I) wherein  $R_4 = -$   
 $CH_2-CO-NH-(D-2-deoxy-mannopyranos-2-yl)$  and the other substituents are  
20 as defined in Example 1].

## EXAMPLE 29

cyclo ([Asn(D-2-deoxy-galactopyranos-2-yl)-Asp-Trp-Phe-Dap-Leu]-

cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 29) [compound of formula I) wherein  $R_4 = -$   
 $CH_2-CO-NH-(D-2-deoxy-galactopyranos-2-yl)$  and the other substituents  
25 are as defined in Example 1].

## EXAMPLE 30

cyclo ([Asn( $\beta$ -D-xylopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))

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(SEQ ID No. 30) [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(\beta-D\text{-xylo-pyranosyl})$  and the other substituents are as defined in Example 1].

## EXAMPLE 31

5 cyclo ([Asn(3-sulfo-propionyl)-Asp-Trp-Phe-Dap-Leu]cyclo-(2 $\beta$ -5 $\beta$ ))  
(SEQ ID 31) [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(3\text{-sulfo-propionyl})$  and the other substituents are as defined in Example 1].

## EXAMPLE 32

cyclo ([Dap(Lysyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 32)  
10 [compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(Lysyl)$  and the other substituents are as defined in Example 1].

## EXAMPLE 33

cyclo ([Dap(Arginyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 33)  
[compound of formula I) wherein  $R_4 = -CH_2-CO-NH-(Arginyl)$  and the  
15 other substituents are as defined in Example 1].

## EXAMPLE 34

cyclo ([Dap(4-O- $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo-  
(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 34) [compound of formula I) wherein  $R_4 = -CH_2-CO-$   
NH-(4-O- $\beta$ -D-galactopyranosyl) and the other substituents are as  
20 defined in Example 1].

## EXAMPLE 35

cyclo ([Asn(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-  
Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 35) [compound of formula I) wherein  $R_4 =$   
-CH<sub>2</sub>-CO-NH-(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc) and the other  
25 substituents are as defined in Example 1].

BIOLOGICAL ACTIVITY

The capability of the compounds of the present invention to interact

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as agonists or antagonists with the neurokynin A (NKA) receptor has been valued in a in vitro test using the pulmonary artery of a rabbit (RPA) (Rovero et al., Neuropeptides, 1989, 13, 263-270) and their activity was determined as  $pK_B$  (antilogarithm of the dissociation constant), as described in Jenkinson et al., TiPS, 12, 53-56, 1991. For example, compound 2 has shown a  $pK_B = 8.67$ . The capability of the products of the present invention to interact as agonists or antagonists with NKA receptor has been valued in vivo as capability, after intravenous administration, to inhibit the agonist [betaAla<sup>8</sup>] NKA (4-10)-induced contractions of the urinary bladder in the anaesthetized mouse, as described in Maggi et al., J. Pharmacol. Exp. Ther., 1991, 257, 1172. Compound 1, e.g., causes, at dose of 10 nmol/Kg i.v., an inhibitory effect of 50-70 %, as it has been valued at different times. The effect lasts over a period of more than 3 hours.

## ABBREVIATIONS:

Asn( $\beta$ -D-Glc): N<sup>G</sup>-( $\beta$ -D-glucopiranosyl)-L-asparagine

Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]: N<sup>G</sup>-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopiranosyl)-L-asparagine

20 Fmoc-Asn[(Ac<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp: N<sup>G</sup>-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopiranosyl)N<sup>a</sup>-(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl esthere

Ser( $\beta$ -D-Glc): O<sup>G</sup>-( $\beta$ -D-glucopiranosyl)L-asparagine

Ser[(Bz<sub>4</sub>O)- $\beta$ -D-Glc]: O<sup>G</sup>-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-glucopiranosyl)L-asparagine

25 Fmoc-Ser[(Bz<sub>4</sub>O)- $\beta$ -D-Glc]-OPfp: O<sup>G</sup>-(2,3,4,6-tetra-o-benzoyl- $\beta$ -D-

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glucopiranosyl)N<sup>a</sup>-(fluoren-9-ylmethoxycarbonyl)-L-serine  
pentafluorophenyl ester.

Glc: glucopyranosyl



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

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(ii) TITLE OF INVENTION: Bicyclic compounds, preparation thereof  
and use in pharmaceutical compositions

(iii) NUMBER OF SEQUENCES: 35

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: IT FI 95 A 000044
- (B) FILING DATE: 13-MAR-1995

## (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

## (2) INFORMATION FOR SEQ ID NO: 1:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-Glc), wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Trp Phe Xaa Leu  
1                    5

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-amino-Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cycle

( 1x ) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

- ```

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 6 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 1
  (D) OTHER INFORMATION: Xaa is Nle, i.e. norleucine

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 5
  (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 6
  (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-2-acetamido
                        -Glc), wherein Glc is glucopyranosyl

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 1 and 6
  (D) OTHER INFORMATION: Nle and Asn are bound together to
                        form a first cyclo

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 2 and 5
  (D) OTHER INFORMATION: Asp and Dap are bound together to
                        form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Xaa Asp Trp Phe Xaa Leu
1          5

```

(2) INFORMATION FOR SEQ ID NO: 6:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-ribofuranosyl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-ribofuranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Ser and Leu are bound together to form a first cycle

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ser Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -L-arabinofuranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -L-arabinofuranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic



(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-mannopyranosyl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-mannopyranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ser Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-galactopyranosyl)

( 1x ) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cycle

( 1x ) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

```
(ix) FEATURE:
      (A) NAME/KEY: Modified-site
      (B) LOCATION: 5
      (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic
```

```
(1x) FEATURE:
      (A) NAME/KEY: Modified-site
      (B) LOCATION: 1
      (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-galactopyranosyl)
```

```
(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 1 and 6
  (D) OTHER INFORMATION: Ser and Leu are bound together to
                        form a first cyclo
```

```
(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 2 and 5
  (D) OTHER INFORMATION: Asp and Dap are bound together to
                        form a second cycle
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ser Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-glucuronopyranosyl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
 (B) LOCATION: 2 and 5  
 (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Ser is Ser( $\beta$ -D-glucuronopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Ser and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ser Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(1-deoxy-sorbitol-1-yl)

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cycle

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cycle

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

( 1x ) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Asn is Asn[4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc],  
wherein Glc is glucopyranosyl

(ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn[4-O-( $\beta$ -D-galactopyranosyl  
- $\beta$ -D-Glc]

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn[O- $\alpha$ -D-Glc-(1-4)-O- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc], wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Asp Trp Phe Xaa Leu  
1                    5

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic



(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-glucopyranos-2-yl)

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| Asn | Asp | Trp | Phe | Xaa | Leu |
| 1   |     |     |     | 5   |     |

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Dap[D(-)-quinyll]

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap[D(-)-quinyl] and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 22:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap[D-gluconyl]

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap[D-gluconyl] and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Xaa is Dap[D-glucuryl]

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Dap[D-glucuryl] and Leu are bound together to form a first cyclo

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 1
  - (D) OTHER INFORMATION: Xaa is Dap(sulfo-benzoyl)

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 5
  - (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 1 and 6
  - (D) OTHER INFORMATION: Dap(sulfo-benzoyl) and Leu are bound together to form a first cyclo

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 2 and 5
  - (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(4-sulfo-phenyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -L-Glc), wherein Glc is glucopyranosyl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Asn Asp Trp Phe Xaa Leu  
1                    5

## (2) INFORMATION FOR SEQ ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-mannopyranos-2-yl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 1  
 (D) OTHER INFORMATION: Asn is Asn(D-2-deoxy-galactopyranos  
 2-yl

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 5  
 (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 1 and 6  
 (D) OTHER INFORMATION: Asn and Leu are bound  
 together to form a first cyclo

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 2 and 5  
 (D) OTHER INFORMATION: Asp and Dap are bound together to  
 form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Asn Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide



## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn( $\beta$ -D-xylopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Asn is Asn(3-sulfo-propionyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Asn Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 32:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap(Lysyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap(Lysyl) and Leu are bound together to form a first cyclo

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Xaa Asp Trp Phe Xaa Leu  
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: bicyclic

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: Xaa is Dap(Arginyl)

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1 and 6  
(D) OTHER INFORMATION: Dap(Arginyl) and Leu are bound together to form a first cyclo

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2 and 5  
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 34:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: Xaa is Dap(4-O- $\beta$ -D-galactopyranosyl)

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 6
- (D) OTHER INFORMATION: Dap(4-O- $\beta$ -D-galactopyranosyl) and Le

are bound together to form a first cycl

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2 and 5
- (D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Xaa Asp Trp Phe Xaa Leu  
1 5

## (2) INFORMATION FOR SEQ ID NO: 35:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: bicyclic

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: Asn is Asn(2-deoxy-2-trifluoro-acetoamido- $\beta$ -D-Glc, wherein Glc is glucopyranosyl

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Dap, i.e. diamino propionic

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1 and 6

(D) OTHER INFORMATION: Asn and Leu are bound together to form a first cyclo

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2 and 5

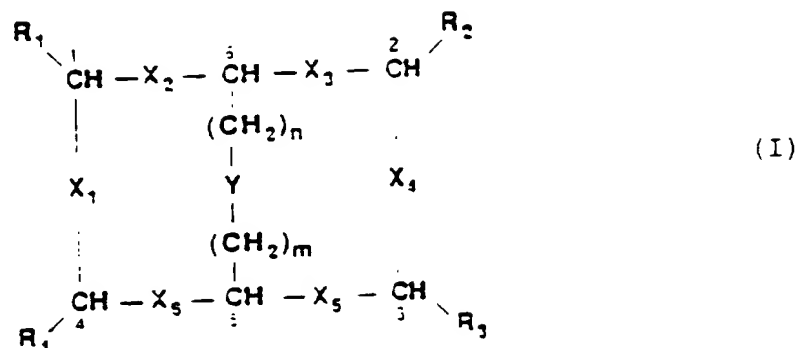
(D) OTHER INFORMATION: Asp and Dap are bound together to form a second cyclo

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| Xaa | Asp | Trp | Phe | Xaa | Leu |
| 1   |     |     |     | 5   |     |

## CLAIMS

1. Bicycl compounds of general Formula



- wherein  $\text{X}_1$ ,  $\text{X}_2$ ,  $\text{X}_3$ ,  $\text{X}_4$ ,  $\text{X}_5$  and  $\text{X}_6$ , same or different from one another, represent a  $-\text{NR}'\text{CO}-$  or a  $-\text{CONR}'-$  group, where  $\text{R}'$  is H or  $\text{C}_{1-3}$  alkyl; Y represents a group selected from  $-\text{NR}\text{CO}-$ ,  $-\text{CONR}-$  or  $-\text{SS}-$  wherein R is H or  $\text{C}_{1-3}$  alkyl; at least one of  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$  groups, same or different from one another, is hydrophilic and the remaining groups are hydrophobic; m and n, same or different from one another, are each an integer number from 1 to 4.
2. Compounds as claimed in claim 1, wherein the hydrophobic groups can be separately selected from the following:
- a) groups corresponding to  $\text{C}_n\text{H}_{2n+1}$  wherein  $n = 0, 1-4$ ;
  - b) linear or branched-alkyl groups corresponding to  $\text{C}_n\text{H}_{2n}-\text{U}-\text{W}$  wherein  $n = 1-4$ ;  $\text{U} = \text{O}$ ,  $\text{COO}$ ,  $\text{CONH}$ , S and  $\text{W} = \text{alkyl-}$ ,  $\text{aryl-}$  or  $\text{alkylaryl-group}$  containing from 1 to 15 C atoms;
  - c)  $(\text{CH}_2)_n-\text{C}_6\text{H}_3-\text{A}-\text{B}$  wherein  $n = 0, 1-3$ ; A and B, placed in any of the ortho, meta or para positions, same or different from one another, represent H, halogen, OR,  $\text{NHR}$ ,  $\text{NR}_2$ ,  $\text{CH}_3$ , SR wherein R is an  $\text{alkyl-}$ ,  $\text{aryl-}$  or  $\text{alkylaryl-group}$  with less than 10 C atoms;

- 11 d)  $(\text{CH}_2)_n\text{-C}_6\text{H}_{10}\text{R}'$ , wherein  $n = 0, 1-3$  and  $\text{R}' = \text{H}, \text{C}_{1-3}$  alkyl  
12 e)  $(\text{CH}_2)_n\text{-heterocycle}$ , wherein  $n = 0, 1-3$  and by the term heterocyclic  
13 imidazolyl-2-yl, indolyl-3-yl, furanyl-3-yl, piridyl-3-yl, imidazolyl-  
14 3-yl are meant;  
15 f) a  $-(\text{CH}_2)_s\text{-}$  group wherein  $s = 3, 4$ , eventually OH-substituted or  
16 condensed with an aromatic group, which cyclizes with one of the two  
17 adjacent  $\text{X}_{1-6}$  groups in order to produce the side chain of proline,  
18 hydroxyproline, octahydroindol-2-carboxylic acid, tetrahydroiso-  
19 quinolinic acid;  
20 g) the side chain of a natural hydrophobic amino acid;  
21 h) the side chain of a natural hydrophilic amino acid, suitably  
22 substituted in order to render it hydrophobic;  
23 i) the side chain of non-natural hydrophobic amino acids selected from  
24 the group consisting of: norleucine, norvaline, alloisoleucine,  
25 cyclohexylglycine (Chg),  $\alpha$ -amino-n-butyric-acid (Aba),  
26 cyclohexylalanine (Cha), aminophenylbutyric acid (Pba), mono- and di-  
27 substituted phenylalanines in ortho, meta and para positions of the  
28 benzene ring with one or more of the following groups:  $\text{C}_{1-10}$  alkyl,  
29  $\text{C}_{1-10}$  alkoxy, halogen,  $\beta$ -2-thienylalanine,  $\beta$ -3-thienylalanine,  $\beta$ -2-  
30 furanylalanine,  $\beta$ -3-furanylalanine,  $\beta$ -2-piridylalanine,  $\beta$ -3-  
31 piridylalanine,  $\beta$ -4-piridylalanine,  $\beta$ -(1-naphtyl)alanine,  $\beta$ -(2-  
32 naphtyl)alanine, O-alkylated serine-threonine-tyrosine-derivatives,  
33 S-alkyl cysteine, S-alkyl homocysteine, N-alkyl lysine, N-alkyl  
34 ornithine, N-alkyl 2,3 diaminopropionic acid.
- 1 3. Compounds as claimed in claim 2 wherein the side chain of a  
2 hydrophobic amino acid according to paragraph g) is the side chain of  
3 an amino acid selected from the group consisting of: glycine, alanine,

4 valine, leucine, isoleucine, methionine, phenylalanine, tyrosine,  
5 tryptophan, proline, histidine, asparagine, glutamine.

1 4. Compounds as claimed in claim 2, wherein the side chain of an  
2 hydrophilic amino acid suitably substituted according to paragraph (h)  
3 is the side chain of an amino acid selected from the group consisting  
4 of: serine, threonine, cysteine, aspartic acid, glutamic acid, t-  
5 carboxyglutamic acid, arginine, ornithine, lysine.

1 5. Compounds according to Claim 2 wherein the hydrophilic groups are  
2 chosen in the group L-Q wherein L is a chemical bond or a linear or  
3 branched C<sub>1-6</sub> alkyl-group and Q is chosen in the group consisting of:  
4 i) hydroxyl, amine, guanidine, carboxyl, sulfate, phosphonate,  
5 phosphate;  
6 ii) linear, branched or cyclic C<sub>1-6</sub> alkyl chain containing one or more  
7 hydroxyl, amine, guanidine, carboxyl, sulfate, phosphate;  
8 iii) an aromatic group mono-, di- or tri-substituted ortho-, meta-,  
9 para-position with hydroxyl, amino, guanidine, carboxyl, sulfate,  
10 phosphate;  
11 iv) a group M, OM, CONHM, NHCOM wherein M is an hydrophilic group  
12 v) an hydrophilic group according to points i)-iv) protected with  
13 groups which are biologically hydrolyzed reforming an hydrophilic  
14 group.

1 6. Compounds according to Claim 5 wherein the group M is chosen in the  
2 group consisting of:  
3 i) eventually substituted mono-, di-, tri-glycosidic residues;  
4 ii) linear, branched or cyclic C<sub>1-6</sub> alkyl-chains, containing one or  
5 more groups hydroxyl, amine, guanidine, carboxyl, sulfate,  
6 phosphonate, phosphate.



1 7. Compounds of Formula (I) as claimed in claim 6, wherein the  
2 glycosidic residues are selected from the group consisting of:  
3 hexoses or pentoses of D or L series in  $\alpha$  or  $\beta$  configuration, selected  
4 from the group wherein: all C atoms bear a free or protected  
5 hydroxylic group; one or more hydroxyls are substituted by: hydrogen;  
6 an amino or acylamino group; C<sub>6</sub> of hexoses and C<sub>5</sub> of pentoses are  
7 part of a carboxylic group; and wherein the eventually present 2 or 3  
8 glycosidic units are linked by a glycosidic bond of  $\alpha$  or  $\beta$   
9 configuration.

1 8. Compounds of general Formula (I) according to claim 7 selected from  
2 the group consisting of: D or L ribose, D or L arabinose, D or L  
3 xylose, D or L lyxose, D or L allose, D or L altrose, D or L glucose,  
4 D or L mannose, D or L gulose, D or L idose, D or L galactose, D or L  
5 talose, D or L allulose, D or L fructose, D or L sorbose, D or L  
6 tagatose; 5-deoxy-D or L-arabinose, 2-deoxy-D or L-glucose, 2-deoxy-D  
7 or L-galactose, 2-deoxy-D or L-arabinose, 2-deoxy-D or L-ribose, D or  
8 L fucose, D or L ramnose; D-glucosamine, D-mannosamine, D-  
9 galactosamine, daunosamine, acosamine and N-acylate derivatives thereof  
10 with lower fat acids, i.e. containing a N-formylic, acetylic,  
11 propionilic, butyric residue; glucuronic acid, galacturonic acid;  
12 cellobiose, lactose, maltose, D-lactosamine, cellotriose, maltotriose;  
13 tris(hydroxymethyl)methyl, D or L arabitol, D or L erythrol, D or L  
14 perseitol, D or L ribitol, D or L sorbitol, D or L xylitol; or those  
15 from the residue of tartaric acid, glucaric acid, gluconic acid,  
16 bycine, quinic acid, mucic acid, glucosaminic acid.

1 9. Compounds of general Formula (I) according to claim 1, wherein if  
2 one or both R<sub>1</sub> and R<sub>4</sub> groups are hydrophilic, both R<sub>2</sub> and R<sub>3</sub> groups

3 are hydrophobic or viceversa.

1 10. Compounds as claimed in claim 1. as hereinafter indicated:

2 i) cyclo([Asn( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 1)

3 ii) cyclo([Ser( $\beta$ -D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No.

4 2)

5 iii) cyclo ([Asn ( $\beta$ -D-2-deoxy-2-amino-Glc)-Asp-Trp-Phe-Dap-Leu]

6 cyclo (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 3)

7 iv) cyclo ( [Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)-Asp-Trp-Phe-Dap-

8 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 4)

9 v) cyclo([Nle-Asp-Trp-Phe-Dap-Asn( $\beta$ -D-2-deoxy-2-acetamido-Glc)]

10 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID 5)

11 vi) cyclo ([Asn( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

12 (2 $\beta$ -5 $\beta$ )) (SEQ ID 6)

13 vii) cyclo ( [ Ser( $\beta$ -D-ribofuranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo

14 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 7)

15 viii) cyclo([Asn( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

16 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 8)

17 ix) cyclo([Ser( $\beta$ -L-arabinofuranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo

18 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 9)

19 x) cyclo([Asn( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))

20 (SEQ ID No. 10)

21 xi) cyclo([Ser( $\beta$ -D-mannopyranosyl)-Asp-Trp-Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ ))

22 (SEQ ID No. 11)

23 xii) cyclo([Asn( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -

24 5 $\beta$ )) (SEQ ID No. 12)

25 xiii) cyclo([Ser( $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -

26 5 $\beta$ )) (SEQ ID No. 13)

- 27 xiv) cyclo ([Asn( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-  
28 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 14)
- 29 xv) cyclo ([Ser( $\beta$ -D-glucuronopyranosyl)-Asp-Trp-Phe-Dap-Leu]  
30 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 15)
- 31 xvi) cyclo ([Asn(1-deoxy-sorbitol-1-yl)-Asp-Trp-Phe-Dap-Leu]cyclo  
32 (2 $\beta$ -5 $\beta$ )) (SEQ ID No. 16)
- 33 xvii) cyclo ([Asn [(4-O-( $\alpha$ -D-Glc)- $\beta$ -D-Glc)]-Asp-Trp-Phe-Dap-  
34 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 17)
- 35 xviii) cyclo ([Asn[(4-O-( $\alpha$ -D-galactopyranosyl)- $\beta$ -D-Glc)]-Asp-Trp-Phe-  
36 Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 18)
- 37 xix) cyclo ([Asn [O- $\alpha$ -D-Glc-(1-4)-O- $\alpha$ -D-Glc-(1-4)- $\alpha$ -D-Glc]-Asp-Trp-  
38 Phe-Dap-Leu] cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 19)
- 39 xx) cyclo ([Asn(D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-  
40 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 20)
- 41 xxi) cyclo ([Dap[D'-]-quinyl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ  
42 ID No. 21)
- 43 xxii) cyclo ([Dap[D-gluconyl]-Asp-Trp-Phe-Dap-Leu] cyclo (2 $\beta$ -5 $\beta$ )) (SEQ  
44 ID No. 22)
- 45 xxiii)cyclo ([Dap[D-glucuryl]-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ  
46 ID No. 23)
- 47 xxiv) cyclo([Dap(2-sulfo-benzoyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
48 (SEQ ID No. 24)
- 49 xxv) cyclo ([Asn(4-sulfo-phenyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))  
50 (SEQ ID No. 25)
- 51 xxvi) cyclo ([Asn( $\beta$ -L-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID  
52 No. 26)
- 53 xxvii) cyclo ([Asn( $\beta$ -D-2-deoxy-glucopyranos-2-yl)-Asp-Trp-Phe-Dap-

- 54 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 27)
- 55 xxviii) cyclo ([Asn( $\beta$ -D-2-deoxy-mannopyranos-2-yl)-Asp-Trp-Phe-Dap-
- 56 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 28)
- 57 xxix) cyclo ([Asn(D-2-deoxy-galactopyranos-2-yl)-Asp-Trp-Phe-Dap-
- 58 Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 29)
- 59 xxx) cyclo ([Asn( $\beta$ -D-xylopyranosyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ ))
- 60 (SEQ ID No. 30)
- 61 xxxi) cyclo ([Asn(3-sulfo-propionyl)-Asp-Trp-Phe-Dap-Leu]cyclo (2 $\beta$ -
- 62 5 $\beta$ )) (SEQ ID No. 31)
- 63 xxxii) cyclo ([Dap(Lysyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID
- 64 No. 32)
- 65 xxxiii) cyclo ([Dap(Arginyl)-Asp-Trp-Phe-Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID
- 66 No. 33)
- 67 xxxiv) cyclo ([Dap(4-O- $\beta$ -D-galactopyranosyl)-Asp-Trp-Phe-Dap-Leu]
- 68 cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 34)
- 69 xxxv) cyclo ([Asn(2-deoxy-2-trifluoroacetamido- $\beta$ -D-Glc)-Asp-Trp-Phe-
- 70 Dap-Leu]cyclo(2 $\beta$ -5 $\beta$ )) (SEQ ID No. 35).

1 11. Pharmaceutical compositions containing as active principle  
2 compounds of general Formula (I) as claimed in claim 1, combined to  
3 suitable carriers.

1 12. Pharmaceutical compositions according to claim 11 for use as  
2 tachykinins antagonists.

1 13. Pharmaceutical compositions as claimed in claim 12 for treatment  
2 of arthrytis, asthma, inflammations, tumoral growth, gastrointestinal  
3 hypermotility, Huntington's disease, neuritis, neuralgia, hemicrania,  
4 hypertension, urinary incontinence, urticaria, symptoms from carcinoid  
5 syndrome, flu and cold.

1    14. Methods for treatment of arthrytis, asthma, inflammations, tumoral  
2    growth, gastrointestinal hypermotility, Huntington's desease,  
3    neuritis, neuralgia, hemicrania, hypertension, urinary incontinence,  
4    urticaria, symptoms from carcinoid syndrome, flu and cold, all  
5    conditions in which doses comprised between 0.1 and 10 mg/Kg of body  
6    weight of active principle consisting of the products of Formula (I),  
7    according to claim 1, are administered to the patient.

## INTERNATIONAL SEARCH REPORT

In national Application No

PCT/EP 96/01028

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K7/22 C07K7/56 C07K7/64 C07K9/00 A61K38/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                | Relevant to claim No. |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y        | WO,A,93 21227 (MENARINI ET AL.) 28 October 1993<br>cited in the application<br>see the whole document<br>---                                                                                                                      | 1-9,<br>11-14         |
| Y        | INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH,<br>vol. 44, no. 2, August 1994, COPENHAGEN DK,<br>pages 105-111, XP000456585<br>G HÖLZEMANN ET AL.: "Cyclic hexapeptide NK-2 antagonists"<br>see the whole document<br>--- | 1-9,<br>11-14         |
|          | ---<br>-/--                                                                                                                                                                                                                       |                       |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

5 July 1996

Date of mailing of the international search report

25.07.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Masturzo, P

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/01028

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                       | Relevant to claim No. |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | <p>CHEMICAL ABSTRACTS, vol. 122, no. 5,<br/>30 January 1995<br/>Columbus, Ohio, US;<br/>abstract no. 46372p,<br/>C A MAGGI ET AL.: "MEN 10, 627, a novel<br/>polycyclic peptide antagonist of<br/>tachykinin NK-2 receptors"<br/>page 114;<br/>XP002007657<br/>see abstract<br/>&amp; J PHARM EXP THER,<br/>vol. 271, no. 3, 1994,<br/>pages 1489-1500,</p> <p>-----</p> | 1-14                  |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 96/01028

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 14 refers to a method of treatment of the human body the search was carried out and based on the alleged effects of the products.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 96/01028

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| W0-A-9321227                              | 28-10-93            | BG-A- 99110                | 29-09-95            |
|                                           |                     | CZ-A- 9402542              | 12-07-95            |
|                                           |                     | EP-A- 0636146              | 01-02-95            |
|                                           |                     | FI-A- 944838               | 14-10-94            |
|                                           |                     | HU-A- 70189                | 28-09-95            |
|                                           |                     | JP-T- 8500331              | 16-01-96            |
|                                           |                     | NO-A- 943861               | 13-10-94            |
|                                           |                     | SK-A- 124294               | 11-07-95            |
|                                           |                     | ZA-A- 9302644              | 22-10-93            |
| -----                                     |                     |                            |                     |



## PATENT COOPERATION TREATY

PCT



RECD 12 MAY 1999

WIPO

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                 |                                                                                                                                                                        |  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| Applicant's or agent's file reference<br><b>1011PTWO</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                 | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)<br><b>FOR FURTHER ACTION</b>                                       |  |
| International application No.<br><b>PCT/EP98/00599</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | International filing date (day/month/year)<br><b>04/02/1998</b> | Priority date (day/month/year)<br><b>07/02/1997</b>                                                                                                                    |  |
| International Patent Classification (IPC) or national classification and IPC<br><b>C07K5/065</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                 |                                                                                                                                                                        |  |
| Applicant<br><b>MENARINI RICERCHE S.P.A. et al.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                 |                                                                                                                                                                        |  |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 4 sheets.</p>                                                                                                                                                                                                         |                                                                 |                                                                                                                                                                        |  |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"><li>I <input checked="" type="checkbox"/> Basis of the report</li><li>II <input type="checkbox"/> Priority</li><li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li><li>IV <input type="checkbox"/> Lack of unity of invention</li><li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li><li>VI <input type="checkbox"/> Certain documents cited</li><li>VII <input checked="" type="checkbox"/> Certain defects in the international application</li><li>VIII <input type="checkbox"/> Certain observations on the international application</li></ul> |                                                                 |                                                                                                                                                                        |  |
| Date of submission of the demand<br><b>04/09/1998</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                 | Date of completion of this report<br><b>10.05.99</b>                                                                                                                   |  |
| Name and mailing address of the international preliminary examining authority:<br> <b>European Patent Office<br/>D-80298 Munich<br/>Tel. (+49-89) 2399-0 Tx: 523656 epmu d<br/>Fax: (+49-89) 2399-4465</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                 | Authorized officer<br><b>Deffner, C-A</b><br>Telephone No. (+49-89) 2399 8535<br> |  |



# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/00599

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

|          |                     |            |                           |
|----------|---------------------|------------|---------------------------|
| 1,2,4-29 | as originally filed |            |                           |
| 3,3a     | as received on      | 18/11/1998 | with letter of 17/11/1998 |

### Claims, No.:

|               |                     |            |                           |
|---------------|---------------------|------------|---------------------------|
| 1 (part),2-14 | as originally filed |            |                           |
| 1 (part)      | as received on      | 18/11/1998 | with letter of 17/11/1998 |

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

|                               |      |        |      |
|-------------------------------|------|--------|------|
| Novelty (N)                   | Yes: | Claims | 1-14 |
|                               | No:  | Claims |      |
| Inventive step (IS)           | Yes: | Claims | 1-14 |
|                               | No:  | Claims |      |
| Industrial applicability (IA) | Yes: | Claims | 1-14 |
|                               | No:  | Claims |      |



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP98/00599

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2. Citations and explanations

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**





**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/EP98/00599

- 1). Following documents represent relevant state of the art for the subject- matter according to the claims:

(D1) US-A-4703034

(D2) Pept.Chem., Vol.17, pp..7- 12 (1980)

(D3) WO-A-9628467

(D4) EP-A-333174

- 2). Amended claim 1 appears to be novel with respect to (D1) claim 1 in that when present  $R_1/R_2$  have the same meaning as  $R_2/R_3$  in (D1) then present  $R_4/R_3$  differ from  $R_1/R_6$  in (D1). The cyclic Tetra- peptides disclosed in Table 3, page 11, of (D2) are disclaimed in amended claim 1 (Article 33(2) PCT).
- 3). Taking (D4) as closest prior art disclosing linear Di- and Tri- peptide Tachykinin antagonists the problem to be solved by the present application can be defined as the provision of further Tachykinin antagonists.  
With respect to the statement on present page 27 (biological activity, lines 26- 28) this problem appears to be solved by supply of present cyclic compounds.

Having regard to the examples and claim 1 of (D4) it appears that the minimum of structure required for Tachykinin antagonistic activity is the presence of the motif D-Trp-Phe or Trp-Phe for cyclic compounds when turning to (D3), see examples. The same structural motif is present in the ensemble of compounds claimed in present claim 1 (see variables  $R_1$  and  $R_2$ ). The compounds of (D4) are linear whereas the compounds of (D3) are bicyclic which is structurally more restricted and rigid compared to present monocyclic compounds.

The applicant filed two documents Bioorg.Med.Chem.Lett., Vol.6, pp.: 367- 72 (1996) and Br.J.Pharmacol., Vol.100, pp.. 588- 92 (1990) showing linear  $NK_2$  receptor antagonists with the motif D-Trp- D-Trp or D-Trp- Val having similar polarity/hydrophobity compared to Trp- Phe.

Br.J.Pharmacol., Vol.104, p.: 355- 60 (1991) filed by the applicant discloses cyclic  $NK_2$  antagonists of bigger ring size (hexapeptides).



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/EP98/00599

With respect to this prior art it appears that the man skilled in the art looking for compounds solving the above problem could not have expected that principally compounds of the present structure would solve the above problem (Article 33(3) PCT).

- 4). For the assessment of the present claims 12- 14 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.
- 5). The description should be in conformity with amended claims as required by Rule 5.1(a)(iii) PCT.

Documents cited by the applicant and referred to in this report:

Bioorg.Med.Chem.Lett., Vol.6, pp.: 367- 72 (1996)

Br.J.Pharmacol., Vol.100, pp.. 588- 92 (1990)

Br.J.Pharmacol., Vol.104, p.: 355- 60 (1991)



R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, which may be the same or different from one another, represent a hydrogen or C<sub>1-3</sub> alkyl group; with the proviso that when R<sub>1</sub> and R<sub>2</sub> are benzyl or 4-hydroxybenzyl then R<sub>3</sub> and R<sub>4</sub> are not isopropyl.

Also included in the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions containing them.

In view of the presence of chiral centres in the compounds of formula (I), also the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

#### 10 State of the art

The NK-2 receptor of tachykinins is widely expressed in the peripheral nervous system of mammals. One of the various effects produced by the selective stimulation of the NK-2 receptor is the contraction of smooth muscle. Hence antagonists of the NK-2 receptor may be considered agents capable of controlling excessive contraction of smooth muscle in any pathological condition in which the release of tachykinins concurs in the genesis of the corresponding disorder.

In particular, the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, kidney infections and colics may be considered conditions in which the administration of NK-2 antagonists may be effective (E.M. Kudlacz *et al.*, Eur. J. Pharmacol., 1993, 241, 17-25).

In addition, a number of NK-2 antagonists capable of surmounting the haemato-encephalic barrier have shown anxiolytic properties (D.M. Walsh *et al.*, Psychopharmacology, 1995, 121, 186-191).

Cyclic compounds, and in particular cyclic hexapeptides (A.T. McKnight *et al.*, Br. J. Pharmacol., 1991, 104, 355) and bicyclic hexapeptides (V. Pavone *et al.*, WO 93/212227) or cyclic hexapseudopeptides (L. Quartara *et al.*, J. Med. Chem., 1994, 37, 3630; S.L. Harbeson *et al.*, Peptides, Chemistry and Biology. Proceedings of the Twelfth American Peptide Symposium, 1992, 124) are known in the literature for their antagonistic activity towards the NK-2 receptor



3a

of tachykinins. In EP-A-333174 linear di- and tri-peptide compounds comprising the -D-Trp-Phe-moiety are described; such compounds possess tachykinin antagonism.

AMENDED SHEET





32  $R_4$  represents a group chosen from among:

33 - hydrogen or  $C_{1-6}$  alkyl

34 - L-Q, where L is a chemical bond or a linear or branched  $C_{1-6}$  alkyl residue and  
35 Q is a group chosen from among:

36 i) H, OH,  $OR_9$ ,  $NH_2$ ,  $NR_9R_{10}$ , guanidine, sulphate, phosphonate, phosphate,  
37 where  $R_9$  and  $R_{10}$ , which may be the same or different from one another,  
38 represent a hydrogen,  $C_{1-3}$  alkyl group,  $C_{1-3}$ hydroxyalkyl,  $C_{1-3}$ dihydroxyalkyl,  $C_{1-3}$   
39 alkyl- $CONHR_{12}$ ,  $C_{1-3}$ alkyltetrazole,  $C_{1-3}$ alkyl-COOH or wherein  $R_9R_{10}$  joined  
40 together form with the N-atom a saturated 4-6 membered heterocycle possibly  
41 containing a further heteroatom chosen in the group consisting of N, O, S and  
42 wherein  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly protected with one or  
43 more  $C_{1-3}$ -acyl groups or substituted with amino-groups or  $C_{1-3}$ acylamino-  
44 groups;

45 ii) COOH, tetrazole,  $SO_2NH_2$ ,  $SO_2NHCOOR_8$ ,  $CONHR_8$ ,  $NHCOR_8$ , where  $R_8$   
46 represents a linear or cyclic  $C_{1-6}$  alkyl chain containing one or more polar groups  
47 chosen from among the group: OH,  $NH_2$ ,  $NR_{15}R_{16}$ , COOH,  $CONHR_{12}$ ,  $PO_3H$ ,  
48  $SO_3H$ ,  $OR_{11}$  and where  $R_{15}$  and  $R_{16}$ , which may be the same or different from  
49 one another, represent a hydrogen or  $C_{1-3}$  alkyl group, and where  $R_{11}$  is a  $C_{1-3}$   
50 alkyl or  $C_{2-4}$  amino-alkyl chain,  $R_{12}$  is a mono-, di-, tri-glycosidic group possibly  
51 protected with one or more  $C_{1-3}$ acyl groups or substituted with amino-groups or  
52  $C_{1-3}$ acylamino-groups or  $R_{15}R_{16}$  joined together form with the N-atom a  
53 saturated 4-6 membered heterocycle possibly substituted with  $C_{1-3}$ alkyl-groups  
54 or with saturated 4-6 membered heterocycle-groups containing at least an N-  
55 atom;

56 iii)  $COOR_{17}$ ,  $CONHR_{12}$ ,  $OR_{12}$  where  $R_{12}$  is a mono-, di- or tri-glycoside group  
57 possibly protected with one or more  $C_{1-3}$  acyl groups or substituted with amine  
58 or  $C_{1-3}$  acylamine groups and  $R_{17}$  is a group  $R_{12}$  as above defined or a group  
59  $C_{1-3}$ alkyl,  $C_{1-3}$ alkylphenyl, wherein the phenyl-group can be substituted with a  
60 group OH,  $NO_2$ ,  $NH_2$ , CN,  $CH_3$ , Cl, Br;

61  $R_5$ ,  $R_6$ ,  $R_7$ , which may be the same or different from one another, represent a  
62 hydrogen or  $C_{1-3}$  alkyl group; with the proviso that when  $R_1$  and  $R_2$  are benzyl



31a

63 or 4-hydroxybenzyl then  $R_3$  and  $R_4$  are not isopropyl, their pharmaceutically  
64 acceptable salts, their enantiomers and mixture thereof.



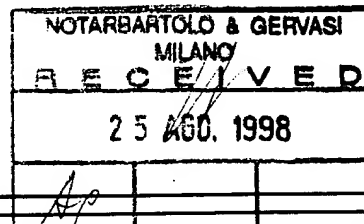
PCT

NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

PASSINI, Angelo  
Notarbartolo & Gervasi S.p.A.  
Corso di Porta Vittoria, 9  
I-20122 Milan  
ITALIE

IMPORTANT NOTICE

|                                                               |                                                                           |                                                               |
|---------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------|
| Date of mailing (day/month/year)<br>13 August 1998 (13.08.98) |                                                                           |                                                               |
| Applicant's or agent's file reference<br>1011PTWO             |                                                                           |                                                               |
| International application No.<br>PCT/EP98/00599               | International filing date (day/month/year)<br>04 February 1998 (04.02.98) | Priority date (day/month/year)<br>07 February 1997 (07.02.97) |
| Applicant<br>MENARINI RICERCHE S.P.A. et al                   |                                                                           |                                                               |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:  
AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,GM,GW,HU,ID,IS,KE,  
KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,  
TM,TR,TT,UA,UG,UZ,VN,YU,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on  
13 August 1998 (13.08.98) under No. WO 98/34949

## REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

## REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

|                                                                                                                                  |                                                                   |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No. (41-22) 740.14.35 | Authorized officer<br>J. Zahra<br>Telephone No. (41-22) 338.83.38 |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|



# PATENT COOPERATION TREATY

PCT

## INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

From the INTERNATIONAL BUREAU

To:

GERVASI, Gemma  
Notarbartolo & Gervasi  
Notarbartolo & Gervasi S.p.A. MILANO  
Corso di Porta Vittoria 8  
I-20122 Milan  
ITALIE

|                       |  |
|-----------------------|--|
| NOTARBAROLO & GERVASI |  |
| RECEIVED              |  |
| - 6 OCT. 1998         |  |
| RG                    |  |

|                                                                  |                                                                           |                                                               |
|------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------|
| Date of mailing (day/month/year)<br>25 September 1998 (25.09.98) |                                                                           |                                                               |
| Applicant's or agent's file reference<br>1011PTWO <i>PLT</i>     |                                                                           |                                                               |
| IMPORTANT INFORMATION                                            |                                                                           |                                                               |
| International application No.<br>PCT/EP98/00599                  | International filing date (day/month/year)<br>04 February 1998 (04.02.98) | Priority date (day/month/year)<br>07 February 1997 (07.02.97) |
| Applicant<br>MENARINI RICERCHE S.P.A. et al                      |                                                                           |                                                               |

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

AP : GH, GM, KE, LS, MW, SD, SZ, UG, ZW

EP : AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

National : AU, BG, BR, CA, CN, CZ, DE, GB, IL, JP, KP, KR, MN, NO, NZ, PL, RO, RU, SE, SK, US,  
VN

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

OA : BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

National : AL, AM, AT, AZ, BA, BB, BY, CH, CU, DK, EE, ES, FI, GE, GH, GM, GW, HU, ID, IS, KE,  
KG, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MW, MX, PT, SD, SG, SI, SL, TJ, TM, TR, TT, UA,  
UG, UZ, YU, ZW

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

|                                                                                               |                                   |
|-----------------------------------------------------------------------------------------------|-----------------------------------|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer:<br>N. Fischer |
| Facsimile No. (41-22) 740.14.35                                                               | Telephone No. (41-22) 338.83.38   |





From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

GERVASI Gemma  
NOTARBARTOLO & GERVASI S.P.A.  
Corso di Porta Vittoria, 9  
I-20122 Milano  
ITALIE



NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)

Date of mailing  
(day/month/year)

10.05.99

Applicant's or agent's file reference  
1011PTWO

IMPORTANT NOTIFICATION

International application No.  
PCT/EP98/00599

International filing date (day/month/year)  
04/02/1998

Priority date (day/month/year)  
07/02/1997

Applicant  
MENARINI RICERCHE S.P.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

 European Patent Office  
D-80298 Munich  
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# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                 |                                                                                                                               |  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|--|
| Applicant's or agent's file reference<br><b>1011PTWO</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                 | <b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |  |
| International application No.<br><b>PCT/EP98/00599</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | International filing date (day/month/year)<br><b>04/02/1998</b> | Priority date (day/month/year)<br><b>07/02/1997</b>                                                                           |  |
| International Patent Classification (IPC) or national classification and IPC<br><b>C07K5/065</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                 |                                                                                                                               |  |
| Applicant<br><b>MENARINI RICERCHE S.P.A. et al.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                 |                                                                                                                               |  |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 4 sheets.</p>                                                                                                                                                                                                                  |                                                                 |                                                                                                                               |  |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the report</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI <input type="checkbox"/> Certain documents cited</li> <li>VII <input checked="" type="checkbox"/> Certain defects in the international application</li> <li>VIII <input type="checkbox"/> Certain observations on the international application</li> </ul> |                                                                 |                                                                                                                               |  |
| Date of submission of the demand<br><b>04/09/1998</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                 | Date of completion of this report<br><b>10.05.99</b>                                                                          |  |
| Name and mailing address of the international preliminary examining authority:<br> European Patent Office<br>D-80298 Munich<br>Tel. (+49-89) 2399-0 Tx: 523656 epmu d<br>Fax: (+49-89) 2399-4465                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                 | Authorized officer<br><br><b>Deffner, C-A</b><br><br>Telephone No. (+49-89) 2399 8535                                         |  |





# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/00599

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

### Description, pages:

|          |                     |            |                |            |
|----------|---------------------|------------|----------------|------------|
| 1,2,4-29 | as originally filed |            |                |            |
| 3,3a     | as received on      | 18/11/1998 | with letter of | 17/11/1998 |

### Claims, No.:

|               |                     |            |                |            |
|---------------|---------------------|------------|----------------|------------|
| 1 (part),2-14 | as originally filed |            |                |            |
| 1 (part)      | as received on      | 18/11/1998 | with letter of | 17/11/1998 |

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

|                               |      |             |
|-------------------------------|------|-------------|
| Novelty (N)                   | Yes: | Claims 1-14 |
|                               | No:  | Claims      |
| Inventive step (IS)           | Yes: | Claims 1-14 |
|                               | No:  | Claims      |
| Industrial applicability (IA) | Yes: | Claims 1-14 |
|                               | No:  | Claims      |



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP98/00599

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**2. Citations and explanations**

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**





**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP98/00599

- 1). Following documents represent relevant state of the art for the subject- matter according to the claims:

(D1) US-A-4703034  
(D2) Pept.Chem., Vol.17, pp..7- 12 (1980)  
(D3) WO-A-9628467  
(D4) EP-A-333174

- 2). Amended claim 1 appears to be novel with respect to (D1) claim 1 in that when present  $R_1/R_2$  have the same meaning as  $R_2/R_3$  in (D1) then present  $R_4/R_3$  differ from  $R_1/R_6$  in (D1). The cyclic Tetra- peptides disclosed in Table 3, page 11, of (D2) are disclaimed in amended claim 1 (Article 33(2) PCT).
- 3). Taking (D4) as closest prior art disclosing linear Di- and Tri- peptide Tachykinin antagonists the problem to be solved by the present application can be defined as the provision of further Tachykinin antagonists.  
With respect to the statement on present page 27 (biological activity, lines 26- 28) this problem appears to be solved by supply of present cyclic compounds.

Having regard to the examples and claim 1 of (D4) it appears that the minimum of structure required for Tachykinin antagonistic activity is the presence of the motif D-Trp-Phe or Trp-Phe for cyclic compounds when turning to (D3), see examples. The same structural motif is present in the ensemble of compounds claimed in present claim 1 (see variables  $R_1$  and  $R_2$ ). The compounds of (D4) are linear whereas the compounds of (D3) are bicyclic which is structurally more restricted and rigid compared to present monocyclic compounds.

The applicant filed two documents Bioorg.Med.Chem.Lett., Vol.6, pp.: 367- 72 (1996) and Br.J.Pharmacol., Vol.100, pp.. 588- 92 (1990) showing linear  $NK_2$  receptor antagonists with the motif D-Trp- D-Trp or D-Trp- Val having similar polarity/hydrophobity compared to Trp- Phe.

Br.J.Pharmacol., Vol.104, p.: 355- 60 (1991) filed by the applicant discloses cyclic  $NK_2$  antagonists of bigger ring size (hexapeptides).



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/EP98/00599

With respect to this prior art it appears that the man skilled in the art looking for compounds solving the above problem could not have expected that principally compounds of the present structure would solve the above problem (Article 33(3) PCT).

- 4). For the assessment of the present claims 12- 14 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.
- 5). The description should be in conformity with amended claims as required by Rule 5.1(a)(iii) PCT.

Documents cited by the applicant and referred to in this report:

Bioorg.Med.Chem.Lett., Vol.6, pp.: 367- 72 (1996)  
Br.J.Pharmacol., Vol.100, pp.. 588- 92 (1990)  
Br.J.Pharmacol., Vol.104, p.: 355- 60 (1991)



$$\begin{array}{c}
 R_5 \quad R_1 \quad R_2 \quad R_6 \\
 | \quad / \quad | \quad / \\
 X_4 - C - X_1 - C - X_2 \\
 | \qquad \qquad \qquad | \\
 (CH_2)_n \qquad \qquad (CH_2)_m \\
 | \qquad \qquad \qquad | \\
 R_4 - CH - (CH_2)_9 - X_3 - (CH_2)_7 - C - R_3 \\
 \qquad \qquad \qquad \qquad \qquad \qquad | \\
 \qquad \qquad \qquad \qquad \qquad \qquad R_7
 \end{array} \tag{I}$$

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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| DK | Denmark                  | LR | Liberia                                  | SG | Singapore                                    |    |                          |
| EE | Estonia                  |    |                                          |    |                                              |    |                          |

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00599

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C07K5/065 C07K7/54

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                   | Relevant to claim No. |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | US 4 703 034 A (FREIDINGER ROGER ET AL)<br>27 October 1987<br>see column 11; claim 1; table IV                                                                                       | 1,2                   |
| X          | KITABATAKE K. ET AL.: "GUSHING- INDUCING<br>PEPTIDES IN BEER PRODUCED BY PENICILLUM<br>CHYRSOGENUM"<br>PEPT.CHEM,<br>vol. 17, 1980, TOKYO,<br>pages 7-12, XP002073620<br>see table 3 | 1,2                   |
| Y          | WO 96 28467 A (MENARINI FARMA IND<br>; ARCAMONE FEDERICO (IT); MAGGI CARLO<br>ALBERTO (I) 19 September 1996<br>see claim 1                                                           | 1-14                  |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

4 August 1998

Date of mailing of the international search report

14/08/1998

Name and mailing address of the ISA  
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Authorized officer

Deffner, C-A

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00599

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                              | Relevant to claim No. |
|----------|---------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y        | EP 0 333 174 A (FUJISAWA PHARMACEUTICAL<br>CO) 20 September 1989<br>see claim 1<br><div style="text-align: center;">-----</div> | 1-14                  |



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/00599

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---|---------------------|----------------------------|---------------------|
| US 4703034                                | A | 27-10-1987          | NONE                       |                     |
| WO 9628467                                | A | 19-09-1996          | IT FI950044 A              | 13-09-1996          |
|                                           |   |                     | AU 5105996 A               | 02-10-1996          |
|                                           |   |                     | BR 9607348 A               | 30-12-1997          |
|                                           |   |                     | CA 2215372 A               | 19-09-1996          |
|                                           |   |                     | CZ 9702862 A               | 18-02-1998          |
|                                           |   |                     | EP 0815126 A               | 07-01-1998          |
|                                           |   |                     | HR 960117 A                | 31-08-1997          |
|                                           |   |                     | NO 974057 A                | 07-11-1997          |
|                                           |   |                     | PL 322105 A                | 05-01-1998          |
|                                           |   |                     | SK 121297 A                | 04-02-1998          |
| EP 0333174                                | A | 20-09-1989          | AT 137763 T                | 15-05-1996          |
|                                           |   |                     | AU 3132489 A               | 21-09-1989          |
|                                           |   |                     | CA 1329444 A               | 10-05-1994          |
|                                           |   |                     | CN 1037156 A               | 15-11-1989          |
|                                           |   |                     | DE 68926403 D              | 13-06-1996          |
|                                           |   |                     | DE 68926403 T              | 17-10-1996          |
|                                           |   |                     | DK 126389 A                | 17-09-1989          |
|                                           |   |                     | FI 891176 A                | 17-09-1989          |
|                                           |   |                     | JP 1287095 A               | 17-11-1989          |
|                                           |   |                     | US 5187156 A               | 16-02-1993          |



## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

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| Date of mailing (day/month/year)<br>25 September 1998 (25.09.98)          |                                                               |
| International application No.<br>PCT/EP98/00599                           | Applicant's or agent's file reference<br>1011PTWO             |
| International filing date (day/month/year)<br>04 February 1998 (04.02.98) | Priority date (day/month/year)<br>07 February 1997 (07.02.97) |
| Applicant<br>GIORGI, Raffaello et al                                      |                                                               |

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
04 September 1998 (04.09.98)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

|                                                                                                                                   |                                                                      |
|-----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>N. Fischer<br>Telephone No.: (41-22) 338.83.38 |
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